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Improved phytases

Phytases are enzymes that hydrolyze phytate (myo-inositol hexakisphosphate) to myo-inositol and inorganic phosphate and are known to be valuable feed additives.

Preferred phytases are myo-inositol hexakisphosphate phosphohydrolases, such as (myo-inositol hexaphosphate 3-phosphohydrolase, EC 3.1.3.8) and (myo-inositol hexaphosphate 6-phosphohydrolase, EC 3.1.3.26).

10 A phytase was first described in rice bran in 1907 [Suzuki et al., Bull. Coll. Agr. Tokio Imp. Univ. 7, 495 (1907)] and phytases from *Aspergillus* species in 1911 [Dox and Golden, J. Biol. Chem. 10, 183-186 (1911)]. Phytases have also been found in wheat bran, plant seeds, animal intestines and in 15 microorganisms [Howsen and Davis, Enzyme Microb. Technol. 5, 377-382 (1983), Lambrechts et al., Biotech. Lett. 14, 61-66 (1992), Shieh and Ware, Appl. Microbiol. 16, 1348-1351 (1968)].

The cloning and expression of the phytase from *Aspergillus niger* (ficcum) has been described by Van Hartingsveldt et al., 20 in Gene, 127, 87-94 (1993) and in European Patent Application, Publication No. (EP) 420 358 and from *Aspergillus niger* var. *awamori* by Piddington et al., in Gene 133, 55-62 (1993).

Cloning, expression and purification of phytases with improved properties have been disclosed in EP 684 313. However, 25 since there is a still ongoing need for further improved phytases, especially with respect to their thermostability, it is an object of the present invention to provide the following process which is, however, not only applicable to phytases.

The present invention relates to improved phytases, viz. 30 phytases of amended characteristics, preferably amended activity characteristics, amended as compared to the phytase(s) it has

been derived from, preferably amended as compared to known phytases. Amended activity characteristics means amended in at least one phytase activity related respect, such as (non-exclusive list): pH stability, temperature stability, pH profile, temperature profile, specific activity (in particular in relation to pH and temperature), substrate specificity, substrate cleavage pattern, substrate binding, position specificity, the velocity and level of release of phosphate from corn, reaction rate, phytate degradation rate), end level of released phosphate reached.

Preferred amended activity characteristics are amended specific activity, preferably increased, and preferably increased at a pH of 3, 4, 5, or 6; amended pH or temperature profile; and/or amended, preferably increased, thermostability, e.g. of an increased melting temperature as measured using DSC.

The present invention also relates to a process for the preparation of a modified protein, wherein in a first step a consensus sequence is determined from a number of highly homologous sequences according to steps a), b) and c) below:

- a) at least three, preferably four amino acid sequences are aligned by any standard alignment program known in the art;
- b) amino acids at the same position according to such alignment are compared regarding their evolutionary similarity by any standard program known in the art, whereas the degree of similarity provided by such a program which defines the least similarity of the amino acids that is used for the determination of an amino acid of corresponding positions is set to a less stringent number and the parameters are set in such a way that it is possible for the program to determine from only 2 identical amino acids at a corresponding position an amino acid for the consensus protein; however, if among the compared amino

acid sequences are sequences that show a much higher degree of similarity to each other than to the residual sequences, these sequences are represented by their consensus sequence determined as defined in the same way as in the present process for the
5 consensus sequence of the consensus protein or a vote weight of 1 divided by the number of such sequences is assigned to every of -those sequences;

c) in case no common amino acid at a defined position is identified by the program, any of the amino acids, preferably
10 the most frequent amino acid of all such sequences is selected;

in a second step the amino acid sequence of another protein which is homologous to the consensus sequence is compared with the consensus sequence; and

in a third step the consensus sequence or the other
15 protein sequence is modified to define a modified sequence;

the modified sequence is back-translated into a DNA sequence, preferably by using a codon frequency table of the organism in which expression should take place;

the DNA sequence is synthesized by methods known in the
20 art and used either integrated into a suitable expression vector or by itself to transform an appropriate host cell;

the transformed host cell is grown under suitable culture conditions and the other protein is isolated from the host cell or its culture medium by methods known in the art.

25 In one aspect of the above process, a modified other protein sequence is defined in the third step as follows: Only those amino acid residues are replaced in the amino acid sequence of the other protein which clearly differ from the consensus sequence of this protein family calculated under
30 moderately stringent conditions whereas at all positions of the alignment where no preferred single amino acid can be determined

under moderately stringent conditions the amino acids of the other protein remain unchanged.

In another aspect, the second step comprises determining the active center of the protein, comprising all amino acid residues that are involved in forming the active center, in the consensus sequence, and in the sequence of the other protein as well; and in the third step a modified consensus sequence is defined as follows: Some or all of the amino acids that form the active center of the other protein are inserted in the backbone of the consensus sequence.

In a preferred process, the program used for the comparison of amino acids at a defined position regarding their evolutionary similarity is the program "PRETTY".

Preferably, the active center of the protein is determined by using an analysis of the three-dimensional structure of the protein.

A preferred homologous protein is an enzyme, a preferred defined protein family is the family of phytases, preferably of fungal origin.

Preferably the amino acid sequence of the phytase is changed by the introduction of at least one mutation selected from the group consisting of

	E58A	F54Y
	D69K	I73V
25	D197N	K94A
	T214L	R101A
	E222T	N153K
	E267D	V158I
	R291I	A203G
30	R329H	S205G
	S364T	V217A

	A379K	A227V
	G404A	V234L
		P238A
		Q277E
5		A287H
		A292Q
		V366I
		A396S
		E415Q
10		G437A
		E451R

whereby the number represents the position in the consensus phytase sequence or a corresponding residue according to an alignment as shown in Fig. 1 when 26 amino acids (signal sequence) are added to the sequences shown in Fig. 1 and the letter before the number represents the amino acid in the phytase which is replaced by the amino acid behind the number.

Preferred host cells are of eukaryotic origin, preferably fungal, such as *Aspergillus*, or yeast, preferably *Saccharomyces* or *Hansenula*.

In another aspect, the invention relates to a modified protein obtainable preferably obtained by any of the above described processes.

In a further aspect, the invention relates to a mutein of the consensus phytase-1, characterized therein that in the amino acid sequence of Figure 2 the following replacements have been effected Q50L, Q50T, Q50G, Q50T-Y51N, Q50L-Y51N or Q50T-K91A.

In a preferred embodiment of this process step b) can also be defined as follows:

b) amino acids at the same position according to such an alignment are compared regarding their evolutionary similarity

by any standard program known in the art, whereas the degree of similarity provided by such program is set at the lowest possible value and the amino acid which is the most similar for at least half of the sequences used for the comparison is
5 selected for the corresponding position in the amino acid sequence of the consensus protein.

By using the above processes the consensus sequence derived from a number of highly homologous sequences can be used in order to replace only certain amino acid residues in the
10 protein in such a manner that only those amino acid residues are replaced which clearly and unambiguously differ from the corresponding consensus sequence of this protein family which has been calculated on moderately stringent conditions. At all other positions of the alignment, however, where the method of
15 the present invention is not able to determine clearly a preferred amino acid residue under moderately stringent conditions the amino acid residues of the other protein are maintained unchanged.

In the alternative, a consensus sequence is determined
20 from homologous sequences as described above. In a second step the active center of the protein comprising all amino acid residues that are involved in forming the active center is determined in the consensus sequence and in the sequence of a single homologous protein as well. The single homologous protein
25 may have preferred properties like high specific activity or different pH dependency of enzymatic activity. In a third step some or all amino acid residues that are involved in forming the active centre of the homologous protein are inserted into the backbone of the consensus sequence. The result thereof is a
30 chimeric protein having the active centre derived from a single protein and the backbone of the consensus sequence.

The active centre of the protein can be determined e.g. by using any analysis of the three-dimensional structure of the protein, e.g. by homology modelling on the basis of a known 3D-structure of a known protein. Frequently the single homologous protein is an enzyme.

It is also an object of the present invention to provide a consensus protein obtainable preferably obtained, by such processes and specifically the consensus protein, which has the amino acid sequences shown in Figures 2, 4 and 6 or a variant thereof. A "variant" refers in the context of the present invention to a consensus protein with amino acid sequence shown in Figure 2, 5, 7, and 8 wherein at one or more positions amino acids have been deleted, added or replaced by one or more other amino acids with the proviso that the resulting sequence provides for a protein whose basic properties like enzymatic activity (type of and specific activity), thermostability, activity in a certain pH-range (pH-stability) have not significantly been changed. "Significantly" means in this context that a man skilled in the art would say that the properties of the variant may still be different but would not be unobvious over the ones of the consensus protein with the amino acid sequence of Figure 2 itself.

A "mutein" refers in the context of the present invention to replacements of the amino acid in the amino acid sequences of the consensus proteins shown in Figure 2 which lead to consensus proteins with further improved properties e.g. activity. Such muteins can be defined and prepared on the basis of the teachings given in European Patent Application number 97810175.6, e. g. Q50L, Q50T, Q50G, Q50L-Y51N, or Q50T-Y51N. "Q50L" means in this context that at position 50 of the amino

acid sequence (Figure 2) the amino acid Q has been replaced by amino acid L.

In addition, a food, feed or pharmaceutical composition comprising a consensus protein as defined above is also an object of the present invention.

In this context "at least three preferably four amino acid sequences of such defined protein family" means that three, four, five, six to 12, 20, 50 or even more sequences can be used for the alignment and the comparison to create the amino acid sequence of the consensus protein. "Sequences of a defined protein family" means that such sequences fold into a three dimensional structure, wherein the alpha-helices, the beta-sheets and beta-turns are at the same position so that such structures are, as called by the man skilled in the art, largely superimposable. Furthermore these sequences characterize proteins which show the same type of biological activity, e.g. a defined enzyme class, e.g. the phytases. As known in the art, the three dimensional structure of one of such sequences is sufficient to allow the modelling of the structure of the other sequences of such a family. An example, how this can be effected, is given in the Reference Example of the present case. "Evolutionary similarity" in the context of the present invention refers to a scheme which classifies amino acids regarding their structural similarity which allows that one amino acid can be replaced by another amino acid with a minimal influence on the overall structure, as this is done e.g. by programs, like "PRETTY", known in the art. The phrase "the degree of similarity provided by such a program...is set to less stringent number" means in the context of the present invention that values for the parameters which determine the degree of similarity in the program used in the practice of the present

invention are chosen in a way to allow the program to define a common amino acid for a maximum of positions of the whole amino acid sequence, e. g. in case of the program PRETTY a value of 2 or 3 for the THRESHOLD and a value of 2 for the PLURALITY can be
5 choosen. Furthermore, "a vote weight of one divided by the number of such sequences" means in the context of the present invention that the sequences which define a group of sequences with a higher degree of similarity as the other sequences used for the determination of the consensus sequence only contribute
10 to such determination with a factor which is equal to one divided by a number of all sequences of this group.

As mentioned before should the program not allow to select the most similar amino acid, the most frequent amino acid is selected, should the latter be impossible the man skilled in the
15 art will select an amino acid from all the sequences used for the comparison which is known in the art for its property to improve the thermostability in proteins as discussed e.g. by Janecek, S. (1993), *Process Biochem.* 28, 435-445 or Fersht, A. R. & Serrano, L. (1993), *Curr. Opin. Struct. Biol.* 3, 75-83.
20 Alber, T. (1989), *Annu. Rev. Biochem.* 58, 765-798 or Matthews, B. W. (1987), *Biochemistry* 26, 6885-6888. Matthews, B. W. (1991), *Curr. Opin. Struct. Biol.* 1, 17-21.

The stability of an enzyme is a critical factor for many industrial applications. Therefore, a lot of attempts, more or
25 less successful, have been made to improve the stability, preferably the thermostability of enzymes by rational (van den Burg et al., 1998) or irrational approaches (Akanuma et al., 1998). The forces influencing the thermostability of a protein are the same as those that are responsible for the proper
30 folding of a peptide strand (hydrophobic interactions, van der Waals interactions, H-bonds, salt bridges, conformational strain

(Matthews, 1993). Furthermore, as shown by Matthews et al. (1987), the free energy of the unfolded state has also an influence on the stability of a protein. Enhancing of protein stability means to increase the number and strength of favorable interactions and to decrease the number and strength of unfavorable interactions. It has been possible to introduce disulfide linkages (Sauer et al, 1986) to replace glycine with alanine residues or to increase the proline content in order to reduce the free energy of the unfolded state (Margarit et al, 1992; Matthews, 1987a). Other groups concentrated on the importance of additional H-bonds or salt bridges for the stability of a protein (Blaber et al, 1993) or tried to fill cavities in the protein interior to increase the buried hydrophobic surface area and the van der Waals interactions (Karpusas et al, 19898). Furthermore, the stabilization of secondary structure elements, especially α -helices, for example, by improved helix capping, was also investigated (Munoz & Serrano, 1995).

However, there is no fast and promising strategy to identify amino acid replacements which will increase the stability, preferably the thermal stability of a protein. Commonly, the 3D structure of a protein is required to find locations in the molecule where an amino acid replacement possibly will stabilize the protein's folded state. Alternative ways to circumvent this problem are either to search for a homologous protein in a thermo- or hyperthermophile organism or to detect stability-increasing amino acid replacements by a random mutagenesis approach. This latter possibility succeeds in only 103 to 104 mutations and is restricted to enzymes for which a fast screening procedure is available (Arase et al, 1993; Risse et al, 1992). For all these approaches, success was

variable and unpredictable and, if successful, the thermostability enhancements nearly always were rather small.

Here we present an alternative way to improve the thermostability of a protein. Imanaka et al (1986) were among the first to use the comparisons of homologous proteins to enhance the stability of a protein. They used a comparison of proteases from thermophilic with homologous ones of mesophilic organisms to enhance the stability of a mesophilic protease. Serrano et al (1993) used the comparison of the amino acid sequences of two homologous mesophilic RNases to construct a more thermostable Rnase. They mutated individually all of the residues that differ between the two and combined the mutations that increase the stability in a multiple mutant. Pantoliano et al (1989) and, in particular, Steipe et al (1994) suggested that the most frequent amino acid at every position of an alignment of homologous proteins contribute to the largest amount to the stability of a protein. Steipe et al (1994) proved this for a variable domain of an immunoglobulin, whereas Pantoliano et al (1989) looked for positions in the primary sequence of subtilisin in which the sequence of the enzyme chosen to be improved for higher stability was singularly divergent. Their approach resulted in the replacement M50F which increased the T_m of subtilisin by 1.8 °C.

Steipe et al. (1994) proved on a variable domain of immunoglobulin that it is possible to predict a stabilizing mutation with better than 60% success rate just by using a statistical method which determines the most frequent amino acid residue at a certain position of this domain. It was also suggested that this method would provide useful results not only for stabilization of variable domains of antibodies but also for domains of other proteins. However, it was never mentioned that

this method could be extended to the entire protein. Furthermore, nothing is said about the program which was used to calculate the frequency of amino acid residues at a distinct position or whether scoring matrices were used as in the present
5 case.

It is therefore an object of the present invention to provide a process for the preparation of a consensus protein comprising a process to calculate an amino acid residue for nearly all positions of a so-called consensus protein and to
10 synthesize a complete gene from this sequence that could be expressed in a pro- or eukaryotic expression system.

DNA sequences of the present invention can be constructed starting from genomic or cDNA sequences coding for proteins, e.g. phytases known in the art [for sequence information see
15 references mentioned above, e.g.

EP 684 313 or sequence data bases, for example like Genbank (Intelligenetics, California, USA), European Bioinformatics Institute (Hinston Hall, Cambridge, GB), NBRF (Georgetown University, Medical Centre, Washington DC, USA) and
20 Vecbase (University of Wisconsin, Biotechnology Centre, Madison, Wisconsin, USA) or disclosed in the figures by methods of in vitro mutagenesis [see e.g. Sambrook et al., Molecular Cloning, Cold Spring Harbor Laboratory Press, New York]. A widely used strategy for "site directed mutagenesis", as originally outlined
25 by Hurchinson and Edgell [J. Virol. 8, 181 (1971)], involves the annealing of a synthetic oligonucleotide carrying the desired nucleotide substitution to a target region of a single-stranded DNA sequence wherein the mutation should be introduced [for review see Smith, Annu. Rev. Genet. 19, 423 (1985) and for
30 improved methods see references 2-6 in Stanssen et al., Nucl. Acid Res., 17, 4441-4454 (1989)]. Another possibility of

mutating a given DNA sequence which is also preferred for the practice of the present invention is the mutagenesis by using the polymerase chain reaction (PCR). DNA as starting material can be isolated by methods known in the art and described e.g. 5 in Sambrook et al. (Molecular Cloning) from the respective strains. For strain information see, e.g. EP 684 313 or any depository authority indicated below. *Aspergillus niger* [ATCC 9142], *Myceliophthora thermophila* [ATCC 48102], *Talaromyces thermophilus* [ATCC 20186] and *Aspergillus fumigatus* [ATCC 34625] 10 have been redeposited according to the conditions of the Budapest Treaty at the American Type Culture Cell Collection under the following accession numbers: ATCC 74337, ATCC 74340, ATCC 74338 and ATCC 74339, respectively. It is however, understood that DNA encoding a consensus protein in accordance 15 with the present invention can also be prepared in a synthetic manner as described, e.g. in EP 747 483 or the examples by methods known in the art.

The process of the present invention can preferably be used in order to improve the thermostability of the enzyme 20 phytase. After having constructed different consensus phytase sequences it was possible to decide whether single amino acid replacements had a positive or a negative effect on the protein stability. It is therefore another subject of the present invention to improve the thermostability of a phytase.

25 In this embodiment single amino acids are changed in the sequence of the phytase by the introduction of at least one mutation selected from the group consisting of

E58A	F54Y
D69K	I73V
30 D197N	K94A
T214L	R101A

	E222T	N153K
	E267D	V158I
	R291I	A203G
	R329H	S205G
5	S364T	V217A
	A379K	A227V
	G404A	V234L
		P238A
		Q277E
10		A287H
		A292Q
		V366I
		A396S
		E415Q
15		G437A
		E451R

In the above-given mutations the number represents the position in the consensus phytase-1-sequence as given in Figure 2 and the letter before the number represents the amino acid in the phytase which is replaced by the respective amino acid behind the number. The numbers given correspond to the consensus phytase sequence or relate to a corresponding residue calculated by an alignment as shown in Figure 1 when 26 amino acids (signal sequence) are added to the sequences shown in Fig. 1. Those mutations can be introduced into consensus sequences or into sequences of specific enzymes which have been improved by a process of the present invention. The above-mentioned amino acid replacements have a positive effect on the protein stability.

Once complete DNA sequences of the present invention have been obtained they can be integrated into vectors by methods

known in the art and described e.g. in Sambrook et al. (s.a.) to overexpress the encoded polypeptide in appropriate host systems. However, a man skilled in the art knows that also the DNA sequences themselves can be used to transform the suitable host systems of the invention to get overexpression of the encoded polypeptide. Appropriate host systems are for example fungi, like *Aspergilli*, e.g. *Aspergillus niger* [ATCC 9142] or *Aspergillus ficuum* [NRRL 3135] or like *Trichoderma*, e.g. *Trichoderma reesei* or yeasts, like *Saccharomyces*, e.g. *Saccharomyces cerevisiae* or *Pichia*, like *Pichia pastoris*, or *Hansenula polymorpha*, e.g. *H. polymorpha* (DSM5215) or plants, as described, e.g. by Pen et al., *Bio/Technology* 11, 811-814 (1994). A man skilled in the art knows that such microorganisms are available from depository authorities, e.g. the American Type Culture Collection (ATCC), the Centraalbureau voor Schimmelcultures (CBS) or the Deutsche Sammlung für Mikroorganismen und Zellkulturen GmbH (DSM) or any other depository authority as listed in the Journal "Industrial Property" [(1991) 1, pages 29-40]. Bacteria which can be used are e.g. *E. coli*, *Bacilli* as, e.g. *Bacillus subtilis* or *Streptomyces*, e.g. *Streptomyces lividans* (see e.g. Anné and Mallaert in *FEMS Microbiol. Letters* 114, 121 (1993). *E. coli*, which could be used are *E. coli* K12 strains e.g. M15 [described as DZ 291 by Villarejo et al. in *J. Bacteriol.* 120, 466-474 (1974)], HB 101 [ATCC No. 33694] or *E. coli* SG13009 [Gottesman et al., *J. Bacteriol.* 148, 265-273 (1981)].

Vectors which can be used for expression in fungi are known in the art and described e.g. in EP 420 358, or by Cullen et al. [*Bio/Technology* 5, 369-376 (1987)] or Ward in *Molecular Industrial Mycology, Systems and Applications for Filamentous Fungi*, Marcel Dekker, New York (1991), Upshall et al.

[Bio/Technology 5, 1301-1304 (1987)] Gwynne et al. [Bio/Technology 5, 71-79 (1987)], Punt et al. [J. Biotechnol. 17, 19-34 (1991)] and for yeast by Sreekrishna et al. [J. Basic Microbiol. 28, 265-278 (1988), Biochemistry 28, 4117-4125 5 (1989)], Hitzemann et al. [Nature 293, 717-722 (1981)] or in

EP 183 070, EP 183 071, EP 248 227, EP 263 311. Suitable vectors which can be used for expression in *E. coli* are mentioned, e.g. by Sambrook et al. [s.a.] or by Fiers et al. in Proc'd. 8th Int. Biotechnology Symposium" [Soc. Franc. de 10 Microbiol., Paris (Durand et al., eds.), pp. 680-697 (1988)] or by Bujard et al. in Methods in Enzymology, eds. Wu and Grossmann, Academic Press, Inc. Vol. 155, 416-433 (1987) and Stüber et al. in Immunological Methods, eds. Lefkovits and Pernis, Academic Press, Inc., Vol. IV, 121-152 (1990). Vectors 15 which could be used for expression in Bacilli are known in the art and described, e.g. in EP 405 370, Proc'd. Natl. Acad. Sci. USA 81, 439 (1984) by Yansura and Henner, Meth. Enzymol. 185, 199-228 (1990) or EP 207 459. Vectors which can be used for the expression in *H. Polymorpha* are known in the art and described, 20 e.g. in Gellissen et al., Biotechnology 9, 291-295 (1991).

Either such vectors already carry regulatory elements, e.g. promoters, or the DNA sequences of the present invention can be engineered to contain such elements. Suitable promotor elements which can be used are known in the art and are, e.g. 25 for *Trichoderma reesei* the *cbh1*- [Haarki et al., Biotechnology 7, 596-600 (1989)] or the *pkil*-promotor [Schindler et al., Gene 130, 271-275 (1993)], for *Aspergillus oryzae* the *amy*-promotor [Christensen et al., Abstr. 19th Lunteren Lectures on Molecular Genetics F23 (1987), Christensen et al., Biotechnology 6, 1419- 30 1422 (1988), Tada et al., Mol. Gen. Genet. 229, 301 (1991)], for *Aspergillus niger* the *glaA*- [Cullen et al., Bio/Technology 5,

369-376 (1987), Gwynne et al., Bio/Technology 5, 713-719 (1987), Ward in Molecular Industrial Mycology, Systems and Applications for Filamentous Fungi, Marcel Dekker, New York, 83-106 (1991)], alcA- [Gwynne et al., Bio/Technology 5, 718-719 (1987)], suc1-
5 [Boddy et al., Curr. Genet. 24, 60-66 (1993)], aphA- [MacRae et al., Gene 71, 339-348 (1988), MacRae et al., Gene 132, 193-198 (1993)], tpiA- [McKnight et al., Cell 46, 143-147 (1986), Upshall et al., Bio/Technology 5, 1301-1304 (1987)], gpdA- [Punt et al., Gene 69, 49-57 (1988), Punt et al., J. Biotechnol. 17,
10 19-37 (1991)] and the pkiA-promotor [de Graaff et al., Curr. Genet. 22, 21-27 (1992)]. Suitable promotor elements which could be used for expression in yeast are known in the art and are, e.g. the pho5-promotor [Vogel et al., Mol. Cell. Biol., 2050-2057 (1989); Rudolf and Hinnen, Proc. Natl. Acad. Sci. 84, 1340-
15 1344 (1987)] or the gap-promotor for expression in Saccharomyces cerevisiae and for Pichia pastoris, e.g. the aox1-promotor [Koutz et al., Yeast 5, 167-177 (1989); Sreekrishna et al., J. Basic Microbiol. 28, 265-278 (1988)], or the FMD promoter [Hollenberg et al., EPA No. 0299108] or MOX-promotor [Ledeboer
20 et al., Nucleic Acids Res. 13, 3063-3082 (1985)] for H. polymorpha.

Accordingly vectors comprising DNA sequences of the present invention, preferably for the expression of said DNA sequences in bacteria or a fungal or a yeast host and such
25 transformed bacteria or fungal or yeast hosts are also an object of the present invention.

It is also an object of the present invention to provide a system which allows for high expression of proteins, preferably phytases like the consensus phytase of the present invention in
30 Hansenula characterized therein that the codons of the encoding DNA sequence of such a protein have been selected on the basis

of a codon frequency table of the organism used for expression, e.g. yeast as in the present case (see e.g. in Example 3) and optionally the codons for the signal sequence have been selected in a manner as described for the specific case in Example 3.

5 That means that a codon frequency table is prepared on the basis of the codons used in the DNA sequences which encode the amino acid sequences of the defined protein family. Then the codons for the design of the DNA sequence of the signal sequence are selected from a codon frequency table of the host cell used for

10 expression whereby always codons of comparable frequency in both tables are used.

Once such DNA sequences have been expressed in an appropriate host cell in a suitable medium the encoded protein can be isolated either from the medium in the case the protein

15 is secreted into the medium or from the host organism in case such protein is present intracellularly by methods known in the art of protein purification or described in case of a phytase, e.g. in EP 420 358. Accordingly a process for the preparation of a polypeptide of the present invention characterized in that

20 transformed bacteria or a host cell as described above is cultured under suitable culture conditions and the polypeptide is recovered therefrom and a polypeptide when produced by such a process or a polypeptide encoded by a DNA sequence of the present invention are also an object of the present invention.

25 Once obtained the polypeptides of the present invention can be characterized regarding their properties which make them useful in agriculture any assay known in the art and described e.g. by Simons et al. [Br. J. Nutr. 64, 525-540 (1990)], Schöner et al. [J. Anim. Physiol. a. Anim. Nutr. 66, 248-255 (1991)],

30 Vogt [Arch. Geflügelk. 56, 93-98 (1992)], Jongbloed et al. [J. Anim. Sci., 70, 1159-1168 (1992)], Perney et al. [Poultry Sci.

72, 2106-2114 (1993)], Farrell et al., [J. Anim. Physiol. a. Anim. Nutr. 69, 278-283 (1993), Broz et al., [Br. Poultry Sci. 35, 273-280 (1994)] and Dünghoef et al. [Animal Feed Sci. Technol. 49, 1-10 (1994)] can be used.

5 In general the polypeptides of the present invention can be used without being limited to a specific field of application, e.g. in case of phytases for the conversion of inositol polyphosphates, like phytate to inositol and inorganic phosphate.

10 Furthermore the polypeptides of the present invention can be used in a process for the preparation of a pharmaceutical composition or compound food or feeds wherein the components of such a composition are mixed with one or more polypeptides of the present invention. Accordingly compound food or feeds or
15 pharmaceutical compositions comprising one or more polypeptides of the present invention are also an object of the present invention. A man skilled in the art is familiar with their process of preparation. Such pharmaceutical compositions or compound foods or feeds can further comprise additives or
20 components generally used for such purpose and known in the state of the art.

It is furthermore an object of the present invention to provide a process for the reduction of levels of phytate in animal manure characterized in that an animal is fed such a feed
25 composition in an amount effective in converting phytate contained in the feedstuff to inositol and inorganic phosphate.

In the present context, a phytase is an enzyme or polypeptide that has phytase activity. The phytase is preferably purified, viz. at least 85%, preferably at least 86, 87, 88, 89,
30 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% pure. The phytase is preferably isolated. Phytase activity can be determined using

any phytase assay known in the art. A preferred assay is the so-called standard assay herein (see Example 9). A preferred assay temperature is the optimum temperature of the actual phytase, and a preferred assay pH is the optimum pH of the actual phytase. A preferred assay is described in Example 9 herein. Another preferred assay is the FYT assay of example 15 of WO 98/28409, hereby incorporated by reference.

In preferred embodiments, the assay temperature is selected within the range of 20-90°C, more preferably 30-80°C, still more preferably 35-75°C. Preferred assay temperatures are 37°C, 50°C, 60°C, and 70°C.

In further preferred embodiments, the assay pH is selected within the range of pH 2-9, more preferably 3-8, still more preferably 3-6. Preferred assay pH values are 3, 4, 5, 6 and 7.

Amino acid sequence homology (or polypeptide or amino acid homology) is determined as the degree of identity between two sequences. This may suitably be determined by means of computer programs known in the art such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711), see also Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-453, hereby incorporated by reference. In release 9.1, for comparing polypeptide sequences, the Length Weight is set to 0, and the Gap Weight is set to 3.0.

The degree of identity or homology between two DNA (nucleic acid) sequences may be determined by means of computer programs known in the art such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711), see also Needleman, S.B. and Wunsch, C.D., (1970),

Journal of Molecular Biology, 48, 443-453, hereby incorporated by reference. In release 9.1, GAP is used with the following settings for DNA sequence comparison: GAP creation penalty of 50 and GAP extension penalty of 3.

5 Suitable experimental conditions for determining whether a given DNA or RNA sequence hybridizes to a specified nucleotide or oligonucleotide probe involves presoaking of the filter containing the DNA or RNA fragments to examine for hybridization in 5 x SSC (Sodium chloride/Sodium citrate), (J. Sambrook, E.F. 10 Fritsch, and T. Maniatis, 1989, Molecular Cloning, A Laboratory Manual, 2d edition, Cold Spring Harbor, New York) for 10 min, and prehybridization of the filter in a solution of 5 x SSC, 5 x Denhardt's solution (Sambrook et al. 1989), 0.5 % SDS and 100 µg/ml of denatured sonicated salmon sperm DNA (Sambrook et al. 15 1989), followed by hybridization in the same solution containing a concentration of 10 ng/ml of a random-primed (Feinberg, A. P. and Vogelstein, B. (1983) Anal. Biochem. 132:6-13), 32P-dCTP-labeled (specific activity > 1 x 10⁹ cpm/µg) probe for 12 hours at approximately 45°C.

20 The filter is then washed twice for 30 minutes in 2 x SSC, 0.5 % SDS at at least 55°C (low stringency), at at least 60°C (medium stringency), at at least 65°C (medium/high stringency), at at least 70°C (high stringency), or at at least 75°C (very high stringency).

25 Molecules to which the oligonucleotide probe hybridizes under these conditions are detected using an x-ray film.

Before describing the present invention in more detail a short explanation of the Figures enclosed is given below.

Figure 1: Design of the consensus phytase sequence. The letters represent the amino acid residues in the one-letter code. The following sequences were used for the alignment: phyA from *Aspergillus terreus* 9A-1 (Mitchell et al, 1997; from amino acid (aa) 27), phyA from *A. terreus* cbs116.46; (van Loon et al., 1998; from aa 27), phyA from *Aspergillus niger* var. *awamori* (Piddington et al, 1993; from aa 27), phyA from *A. niger* T213; from aa 27), phyA from *A. niger* strain NRRL3135 (van Hartingsveldt et al, 1993; from aa 27), phyA from *Aspergillus fumigatus* ATCC 13073 (Pasamontes et al, 1993; from aa 25), phyA from *A. fumigatus* ATCC 32722 (van Loon et al, 1998; from aa 27), phyA from *A. fumigatus* ATCC 58128 (van Loon et al., 1998; from aa 27), phyA from *A. fumigatus* ATCC 26906 (van Loon et al, 1998; from aa 27), phyA from *A. fumigatus* ATCC 32239 (van Loon et al, 1998; from aa 30), phyA from *Emericella nidulans* (Pasamontes et al, 1997a; from aa 25), phyA from *Talaromyces thermophilus* (Pasamontes et al, 1997a; from aa 24), and phyA from *Myceliophthora thermophila* (Mitchell et al, 1997; from aa 19). The alignment was calculated using the program PILEUP. The location of the gaps was refined by hand. Capitalized amino acid residues in the alignment at a given position belong to the amino acid coalition that establish the consensus residue. In bold, beneath the calculated consensus sequence, the amino acid sequence of the finally constructed consensus phytase (Fcp) is shown. The gaps in the calculated consensus sequence were filled by hand according to principals stated in Example 1.

Figure 2: DNA sequence of the consensus phytase-1 gene (fcp) and of the primers used for the gene construction. The calculated amino acid sequence (Figure 1) was converted into a DNA sequence using the program BACKTRANSLATE (Devereux et al.,

1984) and the codon frequency table of highly expressed yeast genes (GCG program package, 9.0). The signal peptide of the phytase from *A. terreus* cbs.116.46 was fused to the N-terminus. The bold bases represent the sequences of the oligonucleotides used to generate the gene. The names of the respective oligonucleotides are alternately noted above or below the sequence. The underlined bases represent the start and stop codon of the gene. The bases written in italics show the two introduced Eco RI sites.

10

Figure 3: Alignment and consensus sequence of five Basidiomycetes phytases. The letters represent the amino acid residues in the one-letter code. The amino acid sequences of the phytases from *Paxillus involutus*, phyA1 (aa 21) and phyA2 (aa 21, WO 98/28409), *Trametes pubescens* (aa 24, WO 98/28409), *Agrocybe pediades* (aa 19, WO 98/28409), and *Peniophora lycii* (aa 21, WO 98/28409) starting with the amino acid residues mentioned in parentheses, were used for the alignment and the calculation of the corresponding consensus sequence called "Basidio" (Example 2). The alignment was performed by the program PILEPUP. The location of the gaps was refined by hand. The consensus sequence was calculated by the program PRETTY. While a vote weight of 0.5 was assigned to the two *P. involutus* phytases, all other genes were used with a vote weight of 1.0 for the consensus sequence calculation. At positions, where the program was not able to determine a consensus residues, the Basidio sequence contains a dash. Capitalized amino acid residues in the alignment at a given position belong to the amino acid coalition that establish the consensus residue.

30

Figure 4: Design of consensus phytase-10 amino acid sequence. Adding the phytase sequence of *Thermomyces lanuginosa* (Berka et al., 1998) and the consensus sequence of the phytases from five Basidiomycetes to the alignment of Figure 1, an improved consensus sequence was calculated by the program PRETTY. Additionally, the amino acid sequence of *A. niger* T213 was omitted, therefore, using a vote weight of 0.5 for the remaining *A. niger* phytase sequences. For further information see Example 2.

10

Figure 5: DNA and amino acid sequence of consensus phytase-10. The amino acid sequence is written above the corresponding DNA sequence using the one-letter code. The sequence of the oligonucleotides which were used to assemble the gene are in bold letters. The label of oligonucleotides and the amino acids, which were changed compared to those for consensus phytase-1, are underlined and their corresponding triplets are highlighted in small cases. The *fcpl0* gene was assembled from the following oligonucleotides: CP-1, CP-2, CP-3.10, CP-4.10, CP-5.10, CP-6, CP-7.10, CP-8.10, CP-9.10, CP-10.10, CP-11.10, CP-12.10, CP-13.10, CP-14.10, CP-15.10, CP-16.10, CP-17.10, CP18.10, CP-19.10, CP-20.10, CP-21.10, CP-22.10. The newly synthesized oligonucleotides are additionally marked by number 10. The phytase contains the following 32 exchanges: Y54F, E58A, D69K, D70G, A94K, N134Q, I158V, S187A, Q188N, D197N, S204A, T214L, D220E, L234V, A238P, D246H, T251N, Y259N, E267D, E277Q, A283D, R291I, A320V, R329H, S364T, I366V, A379K, S396A, G404A, Q415E, A437G, A463E. The mutations accentuated in bold letters revealed a stabilizing effect on consensus phytase-1 as tested as single mutation in consensus phytase-1.

Figure 6: Alignment for the design of consensus phytase-11. In contrast to the design of consensus phytase-10, for the design of the amino acid sequence of consensus
5 phytase-11, all Basidiomycetes phytases were used as independent sequences using an assigned vote weight of 0.2 for each Basidiomycetes sequence. Additionally, the amino acid sequence of *A. niger* T213 was used in that alignment, again.

10 Figure 7: DNA and amino acid sequence of consensus phytase-1-thermo[8]-Q50T-K91A. The amino acid sequence is written above the corresponding DNA sequence using the one-letter code. The replaced amino acid residues are underlined. The stop codon of the gene is marked by a star (*).

15 Figure 8: DNA and amino acid sequence of consensus phytase-10-thermo[3]-Q50T-K91A. The amino acid sequence is written above the corresponding DNA sequence using the one-letter code. The replaced amino acid residues are underlined.
20 The stop codon of the gene is marked by a star (*).

Figure 9: DNA and amino acid sequence of *A. fumigatus* ATCC 13073 phytase alpha-mutant. The amino acid sequence is written above the corresponding DNA sequence using the one-letter code.
25 The replaced amino acid residues are underlined. The stop codon of the gene is marked by a star (*).

Figure 10: DNA and amino acid sequence of consensus phytase-7. The amino acids are written above the corresponding
30 DNA sequence using the one-letter code. The sequence of the oligonucleotides used to assemble the gene are in bold letters.

Oligonucleotides and amino acids that were exchanged are underlined and their corresponding triplets are highlighted in small cases. The fcp7 gene was assembled from the following oligonucleotides: CP-1, CP-2, CP-3, CP-4.7, CP-5.7, CP-6, CP-7, 5 CP-8.7, CP-9, CP-10.7, CP-11.7, CP-12.7, CP-13.7, CP-14.7, CP-15.7, CP-16, CP-17.7, CP-18.7, CP-19.7, CP-20, CP-21, CP-22. The newly synthesized oligonucleotides are additionally marked by number 7. The phytase contains the following 24 exchanges in comparison to the original consensus phytase: S89D, S92G, A94K, 10 D164S, P201S, G203A, G205S, H212P, G224A, D226T, E255T, D256E, V258T, P265S, Q292H, G300K, Y305H, A314T, S364G, M365I, A397S, S398A, G404A, and A405S.

Figure 11: Differential scanning calorimetry (DSC) of 15 consensus phytase-1 and consensus phytase-10. The protein samples were concentrated to ca. 50-60 mg/ml and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10°C/min was applied up to 95°C. DSC of consensus phytase-10 (upper graph) yielded a melting temperature 20 of 85.4°C, which is 7.3°C higher than the melting point of consensus phytase-1 (78.1°C, lower graph).

Figure 12: Differential scanning calorimetry (DSC) of 25 consensus phytase-10-thermo-Q50T and consensus phytase-10-thermo-Q50T-K91A. The protein samples were concentrated to ca. 50-60 mg/ml and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10°C/min was applied up to 95°C. DSC of consensus phytase-10-thermo-Q50T (upper graph) yielded a melting temperature of 88.6°C, while the 30 melting point of consensus phytase-10-thermo-Q50T-K91A was found at 89.3°C.

Figure 13: Comparison of the temperature optimum between consensus phytase-1, consensus phytase-10 and consensus phytase-10-thermo-Q50T. For the determination of the temperature optimum, the phytase standard assay was performed at a series of temperatures between 37 and 86°C. The diluted supernatant of transformed *S. cerevisiae* strains was used for the determination. The other components of the supernatant showed no influence on the determination of the temperature optimum: Δ , consensus phytase-1; \diamond , consensus phytase-10; \blacksquare , consensus phytase 10-thermo-Q50T.

Figure 14: pH-dependent activity profile and substrate specificity of consensus phytase-10 and its variants thermo-Q50T and thermo-Q50T-K91A. The phytase activity was determined using the standard assay in appropriate buffers (see Example 9) at different pH-values. Graph a) shows the pH-dependent activity profile of consensus phytase-10 (\square), consensus phytase-10-thermo-Q50T (\bullet), and consensus phytase-10-thermo-Q50T-K91A (Δ). Graph b) shows the corresponding substrate specificity tested by replacement of phytate by the indicated compounds in the standard assay; open bars, consensus phytase-10 (grey bars, consensus phytase-10-thermo-Q50T; dark bars, consensus phytase-10-thermo-Q50T-K91A). The numbers correspond to the following compounds: 1, phytate; 2, p-nitrophenyl phosphate; 3, phenyl phosphate; 4, fructose-1,6-bisphosphate; 5, fructose-6-phosphate; 6, glucose-6-phosphate; 7, ribose-5-phosphate; 8, DL-glycerol-3-phosphate; 9, glycerol-2-phosphate; 10, 3-phosphoglycerate; 11, phosphoenolpyruvate; 12, AMP; 13, ADP; 14, ATP.

Figure 15: pH-dependent activity profile and substrate specificity of consensus phytase-1-thermo[8]-Q50T and of consensus phytase-1-thermo[8]-Q50T-K91A. The phytase activity was determined using the standard assay in appropriate buffers (see Example 9) at different pH-values. Graph a) shows the pH-dependent activity profile of the Q50T- (■) and the Q50T-K91A-variant (•). Graph b) shows the corresponding substrate specificities tested by replacement of phytate by the indicated compounds in the standard assay (open bars, consensus phytase-1-thermo[8]-Q50T; filled bars, consensus phytase-1-thermo[8]-Q50T-K91A). The substrates are listed in the legend of Figure 14.

Figure 16: Differential scanning calorimetry (DSC) of consensus phytase-1-thermo[8]-Q50T and consensus phytase-1-thermo[8]-Q50T-K91A. The protein samples were concentrated to ca. 50-60 mg/ml and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10°C/min was applied up to 95°C. DSC of consensus phytase-1-thermo[8]-Q50T (upper graph) showed a melting temperature of 84.7°C, while the melting point of consensus phytase-1-thermo[8]-Q50T-K91A was found at 85.7°C.

Figure 17: Comparison of the temperature optimum between consensus phytase-1, consensus phytase-1-thermo[3] and consensus phytase-1-thermo[8]. For the determination of the temperature optimum, the phytase standard assay was performed at a series of temperatures between 37 and 86°C. Purified protein from the supernatant of transformed *S. cerevisiae* strains was used for the determination. O, consensus phytase-1; □, consensus phytase-1-thermo[3]; ▲, consensus phytase 1-thermo[8].

Figure 18: Comparison of the pH-dependent activity profile and substrate specificity of consensus phytase-1, consensus phytase-7, and of the phytase from *A. niger* NRRL 3135. The phytase activity was determined using the standard assay in appropriate buffers (see Example 9) at different pH-values. Graph a) shows the pH-dependent activity profile of consensus phytase-1 (■), the phytase from *A. niger* NRRL 3135 (○), and of consensus phytase-7 (▲). Graph b) shows the corresponding substrate specificity tested by replacement of phytate by the indicated compounds in the standard assay (black bars, *A. niger* NRRL 3135 phytase; grey bars, consensus phytase-1, dashed bars, consensus phytase-7). The substrates are listed in the legend of Figure 14.

15

Figure 19: Differential scanning calorimetry (DSC) of the phytase from *A. fumigatus* ATCC 13073 and of its stabilized alpha-mutant, which contains the following amino acid exchanges F55Y, V100I, F114Y, A243L, S265P, N294D.

The protein samples were concentrated to ca. 50-60 mg/ml and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10°C/min was applied up to 95°C. DSC of consensus *A. fumigatus* 13073 phytase (upper graph) revealed a melting temperature of 62.5°C, while the melting point of the alpha-mutant was found at 67.0°C.

Figure 20: Comparison of the temperature optimum of *A. fumigatus* 13073 wild-type, its *A. fumigatus* alpha-mutant, and a further stabilized alpha-mutant (E59A-S154N-R329H-S364T-G404A). For the determination of the temperature optimum, the phytase standard assay was performed at a series of temperatures between

37 and 75°C. The diluted supernatant of transformed *S. cerevisiae* strains was used for the determination. The other components of the supernatant showed no influence on the determination of the temperature optimum. O, *A. fumigatus* ATCC

5 13073 phytase; ▲, *A. fumigatus* ATCC 13073 alpha-mutant; □, *A. fumigatus* ATCC 13073 alpha-mutant-(E59A-S154N-R329H-S364T-G404A)-Q27T; ■, *A. fumigatus* ATCC 13073 alpha-mutant-(E59A-S154N-R329H-S364T-G404A)-Q27T-K68A. Q27T and K68A corresponds to consensus phytase-1 Q50T and K91A, respectively.

10

Figure 21: Amino acid sequence of consensus phytase 12 (consphyl2) which contains a number of active site residues transferred from the "basidio" consensus sequence to consensus phytase-10-thermo-Q50T-K91A.

15

Example 1

Consensus phytase-1

The amino acid sequence of consensus phytase-1 (fungal consensus phytase, fcp) was designed and calculated as described
20 in Examples 1-2 of EP 0897985. Table 1 below shows the origin and vote weight of the phytase amino acid sequences used for the design of consensus phytase-1. The consensus phytase-1 sequence was furthermore converted into a DNA sequence as described in Example 3 of EP 0897985, and the consensus phytase-1 gene was
25 constructed and cloned as described in Example 4 of EP 0897985. EP 0897985 is hereby incorporated by reference.

Table 1

Origin and vote weight of the phytase amino acid sequences

30 - phyA from *Aspergillus terreus* 9A-1, aa 27, vote weight 0.5 (Mitchell et al., 1997)

- phyA from *Aspergillus terreus* cbs116.46, aa 27, vote weight 0.5 (van Loon et al., 1998)
- phyA from *Aspergillus niger* var. *awamori*, aa 27, vote weight 0.33 (Piddington et al., 1993)
- 5 - phyA from *Aspergillus niger* T213, aa 27, vote weight 0.33
 - phyA from *Aspergillus niger* strain NRRL3135, aa 27, vote weight 0.33 (van Hartingsveldt et al., 1993)
 - phyA from *Aspergillus fumigatus* ATCC 13073, aa 26, vote weight 0.2 (Pasamontes et al., 1997)
- 10 - phyA from *Aspergillus fumigatus* ATCC 32722, aa 26, vote weight 0.2 (van Loon et al., 1998)
 - phyA from *Aspergillus fumigatus* ATCC 58128, aa 26, vote weight 0.2 (van Loon et al., 1998)
 - phyA from *Aspergillus fumigatus* ATCC 26906, aa 26, vote weight 0.2 (van Loon et al., 1998)
- 15 - phyA from *Aspergillus fumigatus* ATCC 32239, aa 30, vote weight 0.2 (van Loon et al., 1998)
 - phyA from *Emericella nidulans*, aa 25, vote weight 1.0 (Roche Nr. R1288, Pasamontes et al., 1997a)
- 20 - phyA from *Talaromyces thermophilus* ATCC 20186, aa 24, vote weight 1.0 (Pasamontes et al., 1997a)
 - phyA from *Myceliophthora thermophila*, aa 19, vote weight 1.0 (Mitchell et al., 1997)

25

Example 2Design of an improved consensus phytase (consensus phytase-10) amino acid sequence

The alignments used for the design of consensus phytase-10

30 were calculated using the program PILEUP from the Sequence Analysis Package Release 9.0 (Devereux et al., 1984) with the

standard parameter (gap creation penalty 12, gap extension penalty 4). The location of the gaps was refined using a text editor.

The following sequences were used for the alignment of the
5 Basidiomycetes phytases starting with the amino acid (aa)
mentioned in Table 2:

Table 2

Origin and vote weight of five Basidiomycetes phytases used for
10 the calculation of the corresponding amino acid consensus
sequence (basidio)

- phyA1 from Paxillus involutus NN005693, aa 21, vote weight 0.5
(WO 98/28409)
- 15 - phyA2 from Paxillus involutus NN005693, aa 21, vote weight 0.5
(WO 98/28409)
- phyA from Trametes pubescens NN9343, aa 24, vote weight 1.0
(WO 98/28409)
- phyA from Agrocybe pediades NN009289, aa 19, vote weight 1.0
20 (WO 98/28409)
- phyA from Peniophora lycii NN006113, aa 21, vote weight 1.0
(WO 98/28409)

The alignment is shown in Figure 3.

25

In Table 3 the genes, which were used for the performance
of the final alignment, are arranged. The first amino acid (aa)
of the sequence which is used in the alignment is mentioned
behind the organism designation.

30

Table 3

Origin and vote weight of the phytase sequences used for the design of consensus phytase 10

- phyA from *Aspergillus terreus* 9A-1, aa 27, vote weight 0.5
5 (Mitchell et al., 1997)
- phyA from *Aspergillus terreus* cbs116.46, aa 27, vote weight 0.5 (van Loon et al., 1998)
- phyA from *Aspergillus niger* var. *awamori*, aa 27, vote weight 0.5 (Piddington et al., 1993)
- 10 - phyA from *Aspergillus niger* strain NRRL3135, aa 27, vote weight 0.5 (van Hartingsveldt et al., 1993)
- phyA from *Aspergillus fumigatus* ATCC 13073, aa 26, vote weight 0.2 (Pasamontes et al., 1997)
- phyA from *Aspergillus fumigatus* ATCC 32722, aa 26, vote
15 weight 0.2 (van Loon et al., 1998)
- phyA from *Aspergillus fumigatus* ATCC 58128, aa 26, vote weight 0.2 (van Loon et al., 1998)
- phyA from *Aspergillus fumigatus* ATCC 26906, aa 26, vote weight 0.2 (van Loon et al., 1998)
- 20 - phyA from *Aspergillus fumigatus* ATCC 32239, aa 30, vote weight 0.2 (van Loon et al., 1998)
- phyA from *Emericella nidulans* , aa 25, vote weight 1.0 (Roche Nr. R1288, Pasamontes et al., 1997a)
- phyA from *Talaromyces thermophilus* ATCC 20186, aa 24, vote
25 weight 1.0 (Pasamontes et al., 1997a)
- phyA from *Myceliophthora thermophila*, aa 19, vote weight 1.0 (Mitchell et al., 1997)
- phyA from *Thermomyces lanuginosa*, aa 36, vote weight 1.0 (Berka et al., 1998)
- 30 - Consensus sequence of five Basidiomycetes phytases, vote weight 1.0 (Basidio, Figure 3)

The corresponding alignment is shown in Figure 4.

Calculation of the amino acid sequence of consensus-10

5 To improve the alignment, we added the original consensus sequence of five phytases from four different Basidiomycetes, called Basidio, still containing the undefined sequence positions (see Figure 3), nearly all phytase sequences used for calculation of the original consensus phytase and one new
10 phytase sequence from the Ascomycete *Thermomyces lanuginosa* to a larger alignment. Using the consensus sequence of the basidiomycetal phytase sequences, does not pay regard to the diversity among the five amino acid sequences, but pays regard to the common and different amino acid residues between the
15 phytases from the Ascomycetes and the Basidiomycetes.

 We set plurality on 2.0 and threshold on 3. The used vote weight are listed in Table 3. The alignment and the corresponding consensus sequence is presented in Figure 4. The new consensus phytase sequence has 32 different amino acids in
20 comparison to the original consensus phytase. Positions for which the program PRETTY was not able to calculate a consensus amino acid residue were filled according to rules mentioned in Example 1. None of the residues suggested by the program was replaced.

25 Furthermore, we included all Basidiomycetes phytases as single amino acid sequences but assigning a vote weight of 0.2 in the alignment. The corresponding alignment is shown in Figure 6. The calculated consensus amino acid sequence (consensus phytase-11) has the following differences to the sequence of
30 consensus phytase-10. Letter X means that the program was not able to calculate a consensus amino acid; the amino acid in

parenthesis corresponds to the amino acid finally included into the consensus phytase-10.

D35X, X(K)69K, X(E)100E, A101R, Q134N, X(K)153N, X(H)190H, X(A)204S, X(E)220D, E222T, V227A, X(R)271R, H287A, X(D)288D, 5 X(K)379K, X(I)389I, E390X, X(E)415E, X(A)416A, X(R)446L, E463A, whereas the numbering is as in Fig. 5.

We also checked single amino acid replacements suggested by the improved consensus sequences 10 and 11 on their influence on the stability of the original consensus phytase. The approach 10 is described in example 3.

Conversion of consensus phytase-10 amino acid sequence to a DNA sequence

The first 26 amino acid residues of *A. terreus* cbs116.46 15 phytase were used as signal peptide and, therefore, fused to the N-terminus of consensus phytase-10. The used procedure is further described in Example 1.

The resulting sequence of the fcp10 gene is shown in Figure 5.

20

Construction and cloning of the consensus phytase-10 gene (fcp10)

The calculated DNA sequence of fcp10 was divided into oligonucleotides of 85 bp, alternately using the sequence of the 25 sense and the anti-sense strand. Every oligonucleotide overlaps 20 bp with its previous and its following oligonucleotide of the opposite strand. The location of all primers, purchased by Microsynth, Balgach (Switzerland) and obtained in a PAGE-purified form, is indicated in Figure 5.

30

PCR-Reactions

In three PCR reactions, the synthesized oligonucleotides were composed to the entire gene. For the PCR, the High Fidelity Kit from Boehringer Mannheim (Boehringer Mannheim, Mannheim, Germany) and the thermo cycler The ProtokollTM from AMS 5 Biotechnology (Europe) Ltd. (Lugano, Switzerland) was used. The following oligonucleotides were used in a concentration of 0.2 pMol/ml.

- Mix 1.10: CP-1, CP-2, CP-3.10, CP-4.10, CP-5.10, CP-6, CP-7.10, CP-8.10, CP-9.10, CP-10.10
- 10 Mix 2.10: CP-9.10, CP-11.10, CP-12.10, CP-13.10, CP-14.10, CP-15.10, CP-16.10, CP-17.10, CP18.10, CP-19.10, CP-20.10, CP-21.10, CP-22.10

The newly synthesized oligonucleotides are marked by number 10. The phytase contains the following 32 exchanges, 15 which are underlined in Figure 5, in comparison to the original consensus phytase: Y54F, E58A, D69K, D70G, A94K, N134Q, I158V, S187A, Q188N, D197N, S204A, T214L, D220E, L234V, A238P, D246H, T251N, Y259N, E267D, E277Q, A283D, R291I, A320V, R329H, S364T, I366V, A379K, S396A, G404A, Q415E, A437G, A463E.

20 Four short PCR primer were used for the assembling of the oligonucleotides:

CP-a: *Eco RI*
5'-TATATGAATTCATGGGCGTGTTCGTC-3'

25

CP-b:
5'-TGAAAAGTTCATTGAAGGTTTC-3'

30

CP-c.10:
5'-TCTTCGAAAGCAGTACACAAAC-3'

CP-e:

Eco RI

5'-TATATGAATTCTTAAGCGAAAC-3'

5 PCR reaction a: 10 μ l Mix 1.10 (2.0 pmol of each
 oligonucleotide)
 2 μ l nucleotides (10 mM each nucleotide)
 2 μ l primer CP-a (10 pmol/ml)
 2 μ l primer CP-c.10 (10 pmol/ml)
 10,0 μ l PCR buffer
10 0.75 μ l polymerase mixture
 73.25 μ l H₂O

15 PCR reaction b: 10 μ l Mix 2.10 (2.0 pmol of each
 oligonucleotide)
 2 μ l nucleotides (10 mM each nucleotide)
 2 μ l primer CP-b (10 pmol/ml)
 2 μ l primer CP-e (10 pmol/ml)
 10,0 μ l PCR buffer
20 0.75 μ l polymerase mixture (2.6 U)
 73.25 μ l H₂O

Reaction conditions for PCR reaction a and b:

25 step 1 2 min - 45°C
 step 2 30 sec - 72°C
 step 3 30 sec - 94°C
 step 4 30 sec - 52°C
 step 5 1 min - 72°C

Step 3 to 5 were repeated 40-times.

The PCR products (670 and 905 bp) were purified by an agarose gel electrophoresis (0.9% agarose) and a following gel extraction (QIAEX II Gel Extraction Kit, Qiagen, Hilden, Germany). The purified DNA fragments were used for the PCR reaction c.

PCR reaction c:

- 6 µl PCR product of reaction a ≈50 ng)
- 6 µl PCR product of reaction b ≈50 ng)
- 2 µl primer CP-a (10 pmol/ml)
- 2 µl primer CP-e (10 pmol/ml)
- 10,0 µl PCR buffer
- 0.75 µl polymerase mixture (2.6 U)
- 73.25 µl H₂O

15 Reaction conditions for PCR reaction c:

step 1	2 min - 94°C
step 2	30 sec - 94°C
step 3	30 sec - 55°C
step 4	1 min - 72°C

20

Step 2 to 4 were repeated 31-times.

The resulting PCR product (1.4 kb) was purified as mentioned above, digested with Eco RI, and ligated in an Eco RI-digested and dephosphorylated pBsk(-)-vector (Stratagene, La Jolla, CA, USA). 1 µl of the ligation mixture was used to transform E. coli XL-1 competent cells (Stratagene, La Jolla, CA, USA). All standard procedures were carried out as described by Sambrook et al. (1987). The DNA sequence of the constructed gene (fcp10) was checked by sequencing as known in the art.

Example 3

Increasing the thermostability of consensus phytase-1 by
introduction of single mutations suggested by the amino
acid sequence of consensus phytase-10 and consensus
phytase-11

In order to increase the thermostability of homologous genes, it is also possible to test the stability effect of each differing amino acid residue between the protein of interest and the calculated consensus sequence and to combine all stabilizing mutations into the protein of interest. We used the consensus phytase as protein of interest and tested the effect on the protein stability of 34 amino acid residues, differing to consensus phytase 10 and/or 11 as single mutations.

To construct muteins for expression in *A. niger*, *S. cerevisiae*, or *H. polymorpha*, the corresponding expression plasmid containing the consensus phytase gene was used as template for site-directed mutagenesis (see Example 6-8). Mutations were introduced using the "quick exchange™ site-directed mutagenesis kit" from Stratagene (La Jolla, CA, USA) following the manufacturer's protocol and using the corresponding primers. All mutations made and their corresponding primers are summarized in Table 4. Plasmids harboring the desired mutation were identified by DNA sequence analysis as known in the art.

Table 4

Primers used for site-directed mutagenesis of consensus phytase

(Exchanged bases are highlighted in bold. The introduction of a restriction site is marked above the sequence. When a

restriction site is written in parenthesis, the mentioned site was destroyed by introduction of the mutation.)

mutation	Primer set
5	
Kpn I	
Q50T	5' -CACTTGTGGGGTACCTACTCTCCATACTTCTC-3' 5' -GAGAAGTATGGAGAGTAGGTACCCACAAGTG-3'
10	
Y54F	5' -GGTCAATACTCTCCATTCTTCTCTTTGGAAG-3' 5' -CTTCCAAAGAGAAGAATGGAGAGTATTGACC-3'
E58A	5' -CATACTTCTCTTTGGCAGACGAATCTGC-3' 5' -GCAGATTCTGTCTGCCAAAGAGAAGTATG-3'
15	
Aat II	
D69K	5' -CTCCAGACGTCCCAAAGGACTGTAGAGTTAC-3' 5' -GTAACCTCTACAGTCCTTTGGGACGTCTGGAG-3'
20	
Aat II	
D70G	5' -CTCCAGACGTCCCAAGACGGCTGTAGAGTTAC-3' 5' -GTAACCTCTACAGCCGTCTGGGACGTCTGGAG-3'
K91A	5' -GATACCCAACCTTCTTCTGCGTCTAAGGCTTACTCTG-3' 5' -CAGAGTAAGCCTTAGACGCAGAAGAAGTTGGGTATC-3'
25	
Sca I	
A94K	5' -CTTCTAAGTCTAAGAACTACTCTGCTTTG-3' 5' -CAAAGCAGAGTACTTCTTAGACTTAGAAG-3'
30	
A101R	5' -GCTTACTCTGCTTTGATTGAACGGATTCAAAGAACGCTAC-3' 5' -GTAGCGTTCTTTTGAATCCGTTCAATCAAAGCAGAGTAAGC-3'
N134Q	5' -CCATTTCGGTGAACAGCAAATGGTTAACTC-3' 5' -GAGTTAACCATTGCTGTTACCGAATGG-3'
35	
Nru I	
K153N	5' -GATACAAGGCTCTCGCGAGAAACATTGTTC-3' 5' -GGAACAATGTTTCTCGCGAGAGCCTTGTATC-3'
40	
Bss HI	
I158V	5' -GATTGTTCCATTTCGTGCGCGCTTCTGGTTC-3' 5' -GAACCAGAAGCGCGCACGAATGGAACAATC-3'

Bcl I

D197N 5' -CTCCAGTTATTAACGTGATCATTCCAGAAGG-3'
5' -CCTTCTGGAATGATCACGTTAATAACTGGAG-3'

Apa I

5 S187A 5' -GGCTGACCCAGGGGCCCAACCACACCAAGC-3'
5' -GCTTGGTGTGGTTGGGCCCTGGGTCAGCC-3'

Nco I

10 T214L 5' -CACTTTGGACCATGGTCTTTGTACTGCTTTTCG-3'
5' -CGAAAGCAGTACAAAGACCA TGGTCCAAAGTG-3'

Avr II

15 E222T 5' -GCTTTCGAAGACTCTACCC TAGGTGACGACGTTG-3'
5' -CAACGTCGTCACCTAGGGTAGAGTCTTCGAAAGC-3'

V227A 5' -GGTGACGACGCTGAAGCTAACTTCAC-3'
5' -GTGAAGTTAGCTTCAGCGTCGTCACC-3'

Sac II

20 L234V 5' -CTAACTTCACCGCGGTGTTTCGCTCCAG-3'
5' -CTGGAGCGAACACCGCGGTGAAGTTAG-3'

25 A238P 5' -GCTTTGTTTCGCTCCACCTATTAGAGCTAGATTGG-3'
5' -CCAATCTAGCTCTAATAGGTGGAGCGAACAAAGC-3'

Hpa I

30 T251N 5' -GCCAGGTGTTAACTTGACTGACGAAG-3'
5' -TTCGTCAGTCAAGTTAACACCTGGC-3'

Aat II

Y259N 5' -GACGAAGACGTCGTAACTTGATGGAC-3'
5' -GTCCATCAAGTTAACGACGTCTTCGTC-3'

Asp I

35 E267D 5' -GTCCATTTCGACACTGTCGCTAGAACTT C-3'
5' -GAAGTTCTAGCGACAGTGTGGAATGGAC-3'

40 E277Q 5' -CTGACGCTACTCAGCTGTCTCCATTC-3'
5' -GAATGGAGACAGCTGAGTAGCGTCAG-3'

A283D 5' -GTCTCCATTCTGTGATTGTTCACTCAC-3'
5' -GTGAGTGAACAAATCACAGAATGGAGAC-3'

Ksp I

45 H287A 5' -GCTTTGTTCAACGCGGACGAATGGAG-3'
5' -CTCCATTTCGTCGCGGTGAACAAAGC-3'

Bam HI

R291I 5' -CACGACGAATGGATCAATACGACTAC-3'
5' -GTAGTCGTATTGGATCCATTCGTCGTG-3'

5

Bsi WI

Q292A 5' -GACGAATGGAGAGCGTACGACTACTTG-3'
5' -CAAGTAGTCGTACGCTCTCCATTCGTC-3'

10

Hpa I

A320V 5' -GGTGTGGTTTCGTTAACGAATTGATTGC-3'
5' -GCAATCAATTCGTTAACGAAACCAACACC-3'

(Bgl II)

15 R329H 5' -GCTAGATTGACTCACTCTCCAGTTCAAG-3'
5' -CTTGAAGTGGAGAGTGAGTCAATCTAGC-3'

Eco RV

20 S364T 5' -CTCACGACAACACTATGATACTATTTTCTTC-3'
5' -GAAGAAAATAGATATCATAGTGTGTCGTGAG-3'

Nco I

I366V 5' -CGACAACCTCCATGGTTTCTATTTTCTTCGC-3'
5' -GCCAAGAAAATAGAAACCATGGAGTTGTGCG-3'

25

Kpn I

A379K 5' -GTACAACGGTACCAAGCCATTGTCTAC-3'
5' -GTAGACAATGGCTTGGTACCGTTGTAC-3'

30

S396A 5' -CTGACGGTTACGCTGCTTCTTGGAC-3'
5' -GTCCAAGAAGCAGCGTAACCGTCAG-3'

G404A 5' -CTGTTCCATTCGCTGCTAGAGCTTAC-3'
5' -GTAAGCTCTAGCAGCGAATGGAACAG-3'

35

Q415E 5' -GATGCAATGTGAAGCTGAAAAGGAACC-3'
5' -GGTTCCTTTTCAGCTTCACATTGCATC-3'

Sal I

40 A437G 5' -CACGGTTGTGGTGTGACAAGTTGGG-3'
5' -CCCAACTTGTGACACCACAACCGTG-3'

Mun I

45 A463E 5' -GATCTGGTGGCAATTGGGAGGAATGTTTCG-3'
5' -CGAAACATTCCTCCCAATTGCCACCAGATC-3'

and accordingly for other mutations.

The temperature optimum of the purified phytases, expressed in *Saccharomyces cerevisiae* (Example 7), was determined as outlined in Example 9. Table 5 shows the effect on the stability of consensus phytase for each mutation introduced.

Table 5

Stability effect of the individual amino acid replacements in

10 consensus phytase-1

(+ or - means a positive, respectively, negative effect on the protein stability up to 1°C, ++ and -- means a positive, respectively, negative effect on the protein stability between 1 and 3°C; the number 10 or 11 corresponds to the consensus
15 phytase sequence that suggests the amino acid replacement.)

stabilizing		neutral		destabilizing	
mutation	effect	mutation	effect	mutation	effect
E58A (10)	+	D69A	±	Y54F (10)	-
D69K (11)	+	D70G (10)	±	V73I	-
D197N (10)	+	N134Q (10)	±	A94K (10)	-
T214L (10)	++	G186H	±	A101R (11)	-
E222T (11)	++	S187A (10)	±	K153N (11)	-
E267D (10)	+	T214V	±	I158V (10)	--
R291I*	+	T251N (10)	±	G203A	--
R329H (10)	+	Y259N (10)	±	G205S	-
S364T (10)	++	A283D (10)	±	A217V	-
A379K (11)	+	A320V (10)	±	V227A (11)	--
G404A (10)	++	K445T	±	L234V (10)	-
		A463E (10)	±	A238P (10)	--
				E277Q (10)	-
				H287A (11)	-
				Q292A (10)	-
				I366V (10)	-
				S396A (10)	--
				Q415E (11)	-
				A437G (10)	--
				E451R	--

*: This amino acid replacement was found in another round of mutations.

5 We combined eight positive mutations (E58A, D197N, E267D, R291I, R329H, S364T, A379K, G404A) in the consensus phytase using the primers and the technique mentioned above in this example. Furthermore, the mutations Q50T and K91A were introduced which mainly influence the catalytical
10 characteristics of the phytase (see patent application EP 97810175.6 and EP 97112688 as well as Example 9). The DNA and amino acid sequence of the resulting phytase gene (consensus phytase-thermo[8]-Q50T-K91A) is shown in Figure 7. In this way, the temperature optimum and the melting point of the consensus
15 phytase was increased by 7°C (Figure 15, 16, 17).

Using the results of Table 5, we further improved the thermostability of consensus phytase 10 by the following back

mutations K94A, V158I, and A396S that revealed a strong negative influence on the stability of consensus phytase. The resulting protein is phytase-10-thermo [3]. Furthermore, we introduced the mutations Q50T and K91A which mainly influence the catalytical characteristics of consensus phytase (see patent application EP 97810175.6 and EP 97112688 as well as Example 9 and Figure 14 and 15). The resulting DNA and amino acid sequence is shown in Figure 8. The optimized phytase showed a 4°C higher temperature optimum and melting point than consensus phytase 10 (Figure 12 and 13). Furthermore, the phytase has also a strongly increased specific activity with phytate as substrate of 250 U/mg at pH 5.5 (Figure 14).

Example 4

15 Stabilization of the phytase of *A. fumigatus* ATCC 13073 by replacement of amino acid residues with the corresponding consensus phytase-1 and consensus phytase-10 residues

At six typical positions where the *A. fumigatus* 13073 is the only or nearly the only phytase in the alignment of Figure 1 that does not contain the corresponding consensus phytase amino acid residue, the non-consensus amino acid residue was replaced by the consensus one. In a first round, the following amino acids were substituted in *A. fumigatus* 13073 phytase, containing the Q27(24)T substitution and the signal sequence of *A. terreus* cbs.116.46 phytase (see European Patent Application No. 97810175.6 and Figure 9): F55(28)Y, V100(73)I, F114(87)Y, A243(220)L, S265(242)P, N294(282)D.

The numbers in parentheses confer to the numbering of Figure 1. Number 27 in the mutation Q27(24)T refers to the sequence numbering of Anigmature (phytase of *A. niger* (ficum))

NRRL 3135) shown in Fig. 1 of EP 0897010. EP 0897010 is hereby incorporated by reference.

In a second round, four of the seven stabilizing amino acid exchanges (E59A, R329H, S364T, G404A) found in the consensus phytase-10 sequence and, tested as single mutation in consensus phytase-1 (Table 5), were additionally introduced into the *A. fumigatus* α -mutant. Furthermore, the amino acid replacement S126(154)N, shown to reduce the protease susceptibility of the phytase, was introduced. The number 126 in the mutation S126(154)N refers to the sequence Afumature shown in Fig. 1 of EP0897010.

The mutations were introduced as described in example 3 (see Table 6) and expressed as described in example 6 to 8. The resulting *A. fumigatus* 13073 phytase variants were called a - mutant (i.e. the *A. fumigatus* ATCC 13073 phytase with the substitutions Q24T, F28Y, V73I, F87Y, A220L, S242P, N282D) and α -mutant or optimized α -mutant (i.e. the *A. fumigatus* α -mutant having the additional substitutions E59A-S154N-R329H-S364T-G404A). K68A is an additional preferred mutation.

The temperature optimum (60°C, Figure 20) and the melting point (67.0°C, Figure 19) of the *A. fumigatus* 13073 phytase α -mutant was increased by 5°C in comparison to the values of the wild-type (temperature optimum: 55°C, T_m: 60°C). The five additional amino acid replacements further increased the temperature optimum by 3°C (Figure 20).

Table 6

Mutagenesis primers for stabilization of *A. fumigatus* phytase ATCC 13073

Mutation	Primer
F55Y	5'-CACGTACTCGCCATACTTTTCGCTCGAG-3' 5'-CTCGAGCGAAAAGTATGGCGAGTACGTG-3'
5	
E58A	(Xho I) 5'-CCATACTTTTCGCTCGCGGACGAGCTGTCCGTG-3' 5'-CACGGACAGCTCGTCCGCGAGCGAAAAGTAGG-3'
V100I	5'-GTATAAGAAGCTTATTACGGCGATCCAGGCC-3' 5'-GGCCTGGATCGCCGTAATAAGCTTCTTATAC-3'
10	
F114Y	5'-CTTCAAGGGCAAGTACGCCTTTTTGAAGACG-3' 5'-CGTCTTCAAAAAGGCGTACTTGCCCTTGAAG-3'
15 A243L	5'-CATCCGAGCTCGCCTCGAGAAGCATCTTC-3' 5'-GAAGATGCTTCTCGAGGCGAGCTCGGATG-3'
S265P	5'-CTAATGGA TGTGTCCGTTTGATACGGTAG-3' 5'-CTACCGTATCAAACGGACACATGTCCATTAG-3'
20	
N294D	5'-GTGGAAGAAGTACGACTACCTTCAGTC-3' 5'-GACTGAAGGTAGTCGTACTTCTTCCAC-3'
	(Mlu I)
25 R329H	5'-GCCCCGGTTGACGCA TTCGCCAGTGCAGG-3' 5'-CCTGCACTGGCGAATGCGTCAACCGGGC-3'
	Nco I
S364T	5'-CACACGACAACACCATGGTTTCCATCTTC-3' 5'-GAAGATGGAAACCATGGTGTGTGTCGTGTG-3'
30	
G404A	(Bss HI) 5'-GTGGTGCCTTTTCGCCGCGGAGCCTACTTC-3' 5'-GAAGTAGGCTCGCGCGCGAAAGGCACCAC-3'
35	

Example 5

Introduction of the active site amino acid residues of the A. niger NRRL 3135 phytase into the consensus phytase-1

We used the crystal structure of the Aspergillus niger NRRL 3135 phytase to define all active site amino acid residues (see Reference Example and EP 97810175.6). Using the alignment of Figure 1, we replaced the following active site

residues and additionally the not identical adjacent ones of the consensus phytase by that of the *A. niger* phytase:

S89D, S92G, A94K, D164S, P201S, G203A, G205S, H212P, G224A, D226T, E255T, D256E, V258T, P265S, Q292H, G300K, Y305H, 5 A314T, S364G, M365I, A397S, S398A, G404A, and A405S

The new protein sequence consensus phytase -7 was backtranslated into a DNA sequence (Figure 10) as described in Example 1. The corresponding gene (*fcp7*) was generated as described in Example 1 using the following oligonucleotide 10 mixes:

Mix 1.7: CP-1, CP-2, CP-3, CP-4.7, CP-5.7, CP-6, CP-7, CP-8.7, CP-9, CP-10.7

Mix 2.7: CP-9, CP-10.7, CP-11.7, CP-12.7, CP-13.7, CP-14.7, 15 CP-15.7, CP-16, CP-17.7, CP-18.7, CP-19.7, CP-20, CP-21, CP-22.

The DNA sequences of the oligonucleotides are indicated in Figure 3. The newly synthesized oligonucleotides are 20 additionally marked by number 7. After assembling of the oligonucleotides using the same PCR primers as mentioned in Example 1, the gene was cloned into an expression vector as described in Examples 6-8.

The pH-profile determined after expression in *H.* 25 polymorpha and purification was shifted into the acidic range of the pH-spectrum showing an optimum at pH 4.5-5.0 (see Figure 18). The enzyme had a broad pH-optimum reaching at least 60% of its maximum activity from pH 2.5 to pH 6.0. Up to pH 5.0, the profile resembled the profile of the *A. niger* NRRL 3135 phytase. 30 However, below pH 5.0 it lacked the typical low at pH 4.0 of the profile of *A. niger* phytase.

Example 6Expression of the consensus phytase genes in Hansenula polymorpha

5 The phytase expression vectors, used to transform H. polymorpha RB11 (Gellissen et al., 1994), was constructed by inserting the Eco RI fragment of pBsk-fcp or variants thereof into the multiple cloning site of the H. polymorpha expression vector pFPMT121, which is based on an ura3 selection marker from
10 S. cerevisiae, a formate dehydrogenase (FMD) promoter element and a methanol oxidase (MO) terminator element from H. polymorpha. The 5' end of the fcp gene is fused to the FMD promoter, the 3' end to the MOX terminator (Gellissen et al., 1996; EP 0299 108 B). The resulting expression vector are
15 designated pFPMTfcp, pFPMTfcp10, pFPMTfcp7.

The constructed plasmids were propagated in E. coli. Plasmid DNA was purified using standard state of the art procedures. The expression plasmids were transformed into the H. polymorpha strain RP11 deficient in orotidine-5'-phosphate
20 decarboxylase (ura3) using the procedure for preparation of competent cells and for transformation of yeast as described in Gellissen et al. (1996). Each transformation mixture was plated on YNB (0.14% w/v Difco YNB and 0.5% ammonium sulfate) containing 2% glucose and 1.8% agar and incubated at 37 °C.
25 After 4 to 5 days individual transformant colonies were picked and grown in the liquid medium described above for 2 days at 37 °C. Subsequently, an aliquot of this culture was used to inoculate fresh vials with YNB-medium containing 2% glucose. After seven further passages in selective medium, the expression
30 vector integrates into the yeast genome in multimeric form. Subsequently, mitotically stable transformants were obtained by

two additional cultivation steps in 3 ml non-selective liquid medium (YPD, 2% glucose, 10 g yeast extract, and 20 g peptone). In order to obtain genetically homogeneous recombinant strains an aliquot from the last stabilization culture was plated on a selective plate. Single colonies were isolated for analysis of phytase expression in YNB containing 2% glycerol instead of glucose to derepress the *fmd* promoter. Purification of the consensus phytases was done as described in Example 7.

10 Example 7

Expression of the consensus phytase genes in *Saccharomyces cerevisiae* and purification of the phytases from culture supernatant

The consensus phytase genes were isolated from the corresponding Bluescript-plasmid (pBsk-fcp, pBSK-fcp10, pBsk-fcp7) and ligated into the Eco RI sites of the expression cassette of the *Saccharomyces cerevisiae* expression vector pYES2 (Invitrogen, San Diego, CA, USA) or subcloned between the shortened GAPFL (glyceraldehyde-3-phosphate dehydrogenase) promoter and the *pho5* terminator as described by Janes et al. (1990). The correct orientation of the gene was checked by PCR. Transformation of *S. cerevisiae* strains. e. g. INVSc1 (Invitrogen, San Diego, CA, USA) was done according to Hinnen et al. (1978). Single colonies harboring the phytase gene under the control of the GAPFL promoter were picked and cultivated in 5 ml selection medium (SD-uracil, Sherman et al., 1986) at 30°C under vigorous shaking (250 rpm) for one day. The preculture was then added to 500 ml YPD medium (Sherman et al., 1986) and grown under the same conditions. Induction of the *gall* promoter was done according to manufacturer's instruction. After four days of incubation cell broth was centrifuged (7000 rpm, GS3 rotor, 15

min, 5°C) to remove the cells and the supernatant was concentrated by way of ultrafiltration in Amicon 8400 cells (PM30 membranes) and ultrafree-15 centrifugal filter devices (Biomax-30K, Millipore, Bedford, MA, USA). The concentrate (10 5 ml) was desalted on a 40 ml Sephadex G25 Superfine column (Pharmacia Biotech, Freiburg, Germany), with 10 mM sodium acetate, pH 5.0, serving as elution buffer. The desalted sample was brought to 2 M $(\text{NH}_4)_2\text{SO}_4$ and directly loaded onto a 1 ml Butyl Sepharose 4 Fast Flow hydrophobic interaction 10 chromatography column (Pharmacia Biotech, Freiburg, Germany) which was eluted with a linear gradient from 2 M to 0 M $(\text{NH}_4)_2\text{SO}_4$ in 10 mM sodium acetate, pH 5.0. Phytase was eluted in the break-through, concentrated and loaded on a 120 ml Sephacryl S-300 gel permeation chromatography column (Pharmacia Biotech, 15 Freiburg, Germany). Consensus phytase and consensus phytase -7 eluted as a homogeneous symmetrical peak and was shown by SDS-PAGE to be approx. 95% pure.

Example 8

20 Expression of the consensus phytase genes in *Aspergillus niger*

The Bluescript-plasmids pBsk-fcp, pBSK-fcp10, and pBsk-fcp7 were used as template for the introduction of a Bsp HI-site upstream of the start codon of the genes and an Eco RV-site 25 downstream of the stop codon. The Expand™ High Fidelity PCR Kit (Boehringer Mannheim, Mannheim, Germany) was used with the following primers:

Primer Asp-1:

30 *Bsp HI*

5'-TATATCATGAGCGTGTTCGTCGTGCTACTGTTC-3'

Primer Asp-2 used for cloning of fcp and fcp7:

Eco RV

5 3'-ACCCGACTTACAAAGCGAATTCTATAGATATAT-5'

Primer Asp-3 used for cloning of fcp10:

Eco RV

10 3'-ACCCTTCTTACAAAGCGAATTCTATAGATATAT-5'

The reaction was performed as described by the supplier. The PCR-amplified fcp-genes had a new Bsp HI site at the start codon, introduced by primer Asp-1, which resulted in a replacement of the second amino acid residue glycine by serine. Subsequently, the DNA-fragment was digested with Bsp HI and Eco RV and ligated into the Nco I site downstream of the glucoamylase promoter of *Aspergillus niger* (glaA) and the Eco RV site upstream of the *Aspergillus nidulans* tryptophan C terminator (trpC) (Mullaney et al., 1985). After this cloning step, the genes were sequenced to detect possible failures introduced by PCR. The resulting expression plasmids which basically corresponds to the pGLAC vector as described in Example 9 of EP 684 313, contained the orotidine-5'-phosphate decarboxylase gene (pyr4) of *Neurospora crassa* as a selection marker. Transformation of *Aspergillus niger* and expression of the consensus phytase genes was done as described in EP 684 313. The consensus phytases were purified as described in Example 7.

30 Example 9

Determination of phytase activity and of temperature optimum

Phytase activity was determined basically as described by Mitchell et al (1997). The activity was measured in an assay mixture containing 0.5% phytic acid (≈ 5 mM) in 200 mM sodium acetate, pH 5.0. After 15 min of incubation at 37°C, the reaction was stopped by addition of an equal volume of 15% trichloroacetic acid. The liberated phosphate was quantified by mixing 100 μ l of the assay mixture with 900 μ l H₂O and 1 ml of 0.6 M H₂SO₄, 2% ascorbic acid and 0.5% ammonium molybdate. Standard solutions of potassium phosphate were used as reference. One unit of enzyme activity was defined as the amount of enzyme that releases 1 μ mol phosphate per minute at 37°C. The protein concentration was determined using the enzyme extinction coefficient at 280 nm calculated according to Pace et al (1995): consensus phytase, 1.101; consensus phytase 7, 1.068; consensus phytase 10, 1.039.

In case of pH-optimum curves, purified enzymes were diluted in 10 mM sodium acetate, pH 5.0. Incubations were started by mixing aliquots of the diluted protein with an equal volume of 1% phytic acid (≈ 10 mM) in a series of different buffers: 0.4 M glycine/HCl, pH 2.5; 0.4 M acetate/NaOH, pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5; 0.4 M imidazole/HCl, pH 6.0, 6.5; 0.4 M Tris/HCl pH 7.0, 7.5, 8.0, 8.5, 9.0. Control experiments showed that pH was only slightly affected by the mixing step. Incubations were performed for 15 min at 37°C as described above.

For determinations of the substrate specificities of the phytases, phytic acid in the assay mixture was replaced by 5 mM concentrations of the respective phosphate compounds. The activity tests were performed as described above.

For determination of the temperature optimum, enzyme (100 μ l) and substrate solution (100 μ l) were pre-incubated for 5 min at the given temperature. The reaction was started by addition of the substrate solution to the enzyme. After 15 min incubation, the reaction was stopped with trichloroacetic acid and the amount of phosphate released was determined.

The pH-optimum of the original consensus phytase was around pH 6.0-6.5 (70 U/mg). By introduction of the Q50T mutation, the pH-optimum shifted to pH 6.0 (130 U/mg). After introduction of K91A, the pH optimum shifted one pH-unit into the acidic pH-range showing a higher specific activity between pH 2.5 and pH 6.0. That was shown for the stabilized mutants and for consensus phytase-10, too (Figure 14 and 15).

Consensus phytase-7, which was constructed to transfer the catalytic characteristics of the *A. niger* phytase NRRL 3135 into the consensus phytase, had a pH-profile which is shifted into the acidic range of the pH-spectrum showing an optimum between pH 4.5 and 5.0 (see Figure 19). The enzyme had a broad pH-optimum reaching at least 60% of its increased maximum activity from pH 2.5 to pH 6.0. The substrate spectrum, too, resemble more to that of the *A. niger* NRRL 3135 phytase than to the consensus phytase-1.

The temperature optimum of consensus phytase-1 (71°C) was 16-26°C higher than the temperature optimum of the wild-type phytases (45-55°C, Table 7) which were used to calculate the consensus sequence. The improved consensus phytase-10 showed a further increase of its temperature optimum to 80°C (Figure 11).

The temperature optimum of the consensus phytase-1-thermo[8] was found in the same range (78°C) using the supernatant of an overproducing *S. cerevisiae* strain. The

highest temperature optimum reached of 82°C was determined for consensus phytase-10-thermo-Q50T-K91A.

Table 7

- 5 Temperature optimum and T_m-value of consensus phytase and of the phytases from *A. fumigatus*, *A. niger*, *E. nidulans*, and *M. thermophila*.

The determination of the temperature optimum was performed
10 as described in Example 9. The T_m-values were determined by differential scanning calorimetry as described in Example 10.

phytase	temperature optimum [°C]	T _m [°C]
Consensus phytase-10-thermo-Q50T-K91A	82	89.3
Consensus phytase-10-thermo-Q50T	82	88.6
Consensus phytase-10	80	85.4
Consensus phytase-1-thermo[8]-Q50T	78	84.7
Consensus phytase-1-thermo[8]-Q50T-K91A	78	85.7
Consensus phytase-1	71	78.1
<i>A. niger</i> NRRL3135	55	63.3
<i>A. fumigatus</i> 13073	55	62.5
<i>A. fumigatus</i> 13073 α-mutant	60	67.0
<i>A. fumigatus</i> 13073 α-mutant (optimized)	63	-
<i>A. terreus</i> 9A-1	49	57.5
<i>A. terreus</i> cbs.116.46	45	58.5
<i>E. nidulans</i>	45	55.7
<i>M. thermophila</i>	55	n. d.
<i>T. thermophilus</i>	45	n. d.

Example 10

Determination of the melting point by differential scanning calorimetry (DSC)

- 5 In order to determine the unfolding temperature of the phytases, differential scanning calorimetry was applied as previously published by Brugger et al (1997). Solutions of 50-60 mg/ml homogeneous phytase were used for the tests. A constant heating rate of 10°C/min was applied up to 90-95°C.
- 10 The determined melting points reflect the results obtained for the temperature optimums (Table 7). The most stable consensus phytase designed is consensus phytase-10-thermo-Q50T-K91A showing a melting temperature under the choosen condition of 89.3°C. This is 26 to 33.6°C higher than the melting point of
- 15 the wild-type phytases used.

Example 11

Transfer of basidiomycete phytase active site into consensus phytase-10-thermo-Q50T-K91A

- 20 As described previously (Example 3), mutations derived from the basidiomycete phytase active site were introduced into the consensus phytase 10. The following five constructs a) to e) were prepared:

a) This construct is called consensus phytase 12, and it
25 comprises a selected number of active site residues of the basidio consensus sequence, its amino acid sequence (consphyl2) is shown in Fig. 21 (the first 26 amino acids forms the signal peptide, amended positions are underlined);

b) a cluster of mutations (Cluster II) was transferred to
30 the consensus 10 sequence, viz.: S80Q, Y86F, S90G, K91A, S92A, K93T, A94R, Y95I;

c) analogously, another cluster of mutations (Cluster III) was transferred, viz.: T129V, E133A, Q143N, M136S, V137S, N138Q, S139A;

d) analogously, a further cluster of mutations (Cluster IV) was transferred, viz.: A168D, E171T, K172N, F173W;

e) and finally, a further cluster of mutations (Cluster V) was transferred, viz.: Q297G, S298D, G300D, Y305T.

These constructs were expressed as described in Examples 6 to 8.

10

Example 12

Phytase alignment using GAP

The phytases described herein - i.e. the amino acid sequences as well as the corresponding DNA sequences - were aligned against each other. Also some other phytases were correspondingly aligned, viz. the following:

- the consensus phytase described in EP 0897985;
- the phytase derived from *Aspergillus niger* (ficuum) NRRL 3135 (A. niger NRRL 3135) described in EP 0420358;
- 20 ■ the phytases derived from *Aspergillus fumigatus* ATCC 13073 (A. fumigatus 13073); *Aspergillus fumigatus* ATCC 32239 (A. fumigatus 32239); *Aspergillus terreus* CBS 116.46 (A. terreus cbs); *Aspergillus nidulans* (E. nidulans); and *Talaromyces thermophilus* (T. thermophilus) - all described in EP 0897010;
- 25 ■ the phytases derived from *Myceliophthora thermophila* (M. thermophila); and *Aspergillus terreus* 9-A1 (A. terreus 9-A1) - both described in EP 0684313;
- the phytase derived from *Thermomyces lanuginosus* (T. lanuginosus) described in WO 9735017 (PCT/US97/04559);
- 30 ■ the phytases derived from *Agrocybe pediades* (A. pediades), *Paxillus involutus* 1 and 2 (P. involutus 1 and 2); and

Trametes pubescens (T. pubescens) - all described i WO 98/28409; and

- the phytase derived from Peniophora lycii (P. lycii) described in WO 98/28408.

5 For the alignments, the program GAP was used with the settings as described above.

For polypeptide comparisons, the signal peptide were included. However, for alignment to consensus phytase 11, the signal peptides were excluded and only the mature protein part 10 of the other sequences were compared to it.

The results are shown in Table 8 below. The first number in each box or cell is the amino acid similarity, the second number is the amino acid identity.

For DNA sequence comparisons, the signal sequence was 15 included (the same in all phytases).

The results are shown in Table 9 below.

The following embodiments are preferred:

20 Phytases and corresponding DNA sequences related to consensus phytase 10 (CP10, Fcp 10)

A phytase which comprises an amino acid sequence which is at least 93.80%, preferably at least 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of 25 amino acids 1-467 of consensus phytase 10 (Fcp10) as shown in Fig. 5.

A phytase which comprises an amino acid sequence which is at least 95.09%, preferably at least 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-467 30 of consensus phytase 10.

A phytase which is encoded by a DNA sequence which is at least 95.88, preferably at least 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to nucleotides 12-1412 of the DNA sequence of consensus phytase 10 (Fcpl0) as shown in Fig. 5.

5 A DNA sequence which encodes a phytase and which (i) is at least 95.88%, preferably at least 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical, or (ii) hybridizes under low, preferably medium, medium/high, high, very high stringency conditions to nucleotides 12-1412 of the DNA sequence of consensus phytase 10
10 (Fcpl0) as shown in Fig. 5. A preferred negative control is DNA encoding consensus phytase. A preferred positive control is DNA encoding any of CP10, CP10-thermo(3)-Q50T, K91A, CP1-thermo(8), CP1-thermo(8)Q50T, K91A.

A DNA sequence which encodes a phytase comprising an amino
15 acid sequence which is at least 93.80%, preferably at least 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 10 (Fcpl0) as shown in Fig. 5.

20 Phytases and corresponding DNA sequences related to
consensus phytase 10 thermo(3) Q50T, K91A

A phytase which comprises an amino acid sequence which is at least 93.37%, preferably at least 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the
25 sequence of amino acids 1-467 of consensus phytase 10 thermo(3) Q50T, K91A as shown in Fig. 8.

A phytase which comprises an amino acid sequence which is at least 94.66%, preferably at least 95.0, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of amino acids
30 1-467 of consensus phytase 10 thermo(3) Q50T, K91A as shown in Fig. 8.

A phytase which is encoded by a DNA sequence which is at least 95.88, preferably at least 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to nucleotides 12-1412 of the DNA sequence of consensus phytase 10 thermo(3) Q50T, K91A as shown in Fig. 8.

5 A DNA sequence which encodes a phytase and which (i) is at least 95.88%, preferably at least 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical, or (ii) hybridizes under low, preferably medium, medium/high, high, very high stringency conditions to nucleotides 12-1412 of the DNA sequence of consensus phytase 10
10 thermo(3) Q50T, K91A as shown in Fig. 8. A preferred negative control is DNA encoding consensus phytase. A preferred positive control is DNA encoding any of CP10, CP10-thermo(3)-Q50T, K91A, CP1-thermo(8), CP1-thermo(8)Q50T, K91A.

A DNA sequence which encodes a phytase comprising an amino
15 acid sequence which is at least 93.37%, preferably at least 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 10 thermo(3) Q50T, K91A as shown in Fig. 8.

20 Phytases and corresponding DNA sequences related to
consensus phytase 1-thermo(8)

A phytase which comprises an amino acid sequence which is at least 98.30%, preferably at least 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 1
25 thermo(8) (as shown in Fig. 7, backmutations T50Q, A91K to be added).

A phytase which comprises an amino acid sequence which is at least 98.51%, preferably at least 99, 99.5% similar to the sequence of amino acids 1-467 of consensus phytase 1 thermo(8)
30 (as shown in Fig. 7, backmutations T50Q, A91K to be added).

A phytase which is encoded by a DNA sequence which is at least 98.73, preferably at least 98.5, 99, 99.5% identical to nucleotides 1-1407 of the DNA sequence of consensus phytase 1 thermo(8) (as shown in Fig. 7, backmutations T50Q,A91K to be added).

A DNA sequence which encodes a phytase and which (i) is at least 98.73, preferably at least 98.5, 99, 99.5% identical, or (ii) hybridizes under low, preferably medium, medium/high, high, very high stringency conditions to nucleotides 1-1407 of the DNA sequence of consensus phytase 1 thermo(8) (as shown in Fig. 7, backmutations T50Q,A91K to be added). A preferred negative control is DNA encoding consensus phytase. A preferred positive control is DNA encoding any of CP1-thermo(8), CP1-thermo(8)Q50T,K91A.

A DNA sequence which encodes a phytase comprising an amino acid sequence which is at least 98.30%, preferably at least 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 1 thermo(8) (as shown in Fig. 7, backmutations T50Q,A91K to be added).

Phytases and corresponding DNA sequences related to consensus phytase 1 thermo(8) Q50T, K91A

A phytase which comprises an amino acid sequence which is at least 97.87%, preferably at least 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 1 thermo(8) Q50T, K91A as shown in Fig. 7.

A phytase which comprises an amino acid sequence which is at least 98.08%, preferably at least 98.5, 99, 99.5% similar to the sequence of amino acids 1-467 of consensus phytase 1 thermo(8) Q50T, K91A as shown in Fig. 7.

A phytase which is encoded by a DNA sequence which is at least 98.37, preferably at least 98.5, 99, 99.5% identical to nucleotides 1-1407 of the DNA sequence of consensus phytase 1 thermo(8) Q50T, K91A as shown in Fig. 7.

5 A DNA sequence which encodes a phytase and which (i) is at least 98.37, preferably at least 98.5, 99, 99.5% identical, or (ii) hybridizes under low, preferably medium, medium/high, high, very high stringency conditions to nucleotides 1-1407 of the DNA sequence of consensus phytase 1 thermo(8) Q50T, K91A as shown in
10 Fig. 7. A preferred negative control is DNA encoding consensus phytase. A preferred positive control is DNA encoding any of CP1-thermo(8), CP1-thermo(8)Q50T,K91A.

A DNA sequence which encodes a phytase comprising an amino acid sequence which is at least 97.87%, preferably at least 98,
15 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 1 thermo(8) Q50T, K91A as shown in Fig. 7.

Phytases and corresponding DNA sequences related to
consensus phytase 11

20 A phytase which comprises an amino acid sequence which is at least 90.71%, preferably at least 91. 91.5, 92, 92.5, 93, 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-482 of consensus phytase 11 as shown in Fig. 6.

25 A phytase which comprises an amino acid sequence which is at least 92.07%, preferably at least 92.5, 93, 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-482 of consensus phytase 11 as shown in Fig. 6.

30 A DNA sequence which encodes a phytase comprising an amino acid sequence which is at least 90.71%, preferably at least 91.

91.5, 92, 92.5, 93, 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-482 of consensus phytase 11 as shown in Fig. 6.

5 Phytases and corresponding DNA sequences related to A. fumigatus alpha-mutant

A phytase which comprises an amino acid sequence which is at least 97.17%, preferably at least 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of A. fumigatus
10 alpha-mutant (phytase) as shown in Fig. 9.

A phytase which comprises an amino acid sequence which is at least 97.82%, preferably at least 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-467 of A. fumigatus alpha-mutant (phytase) as shown in Fig. 9.

15 A phytase which is encoded by a DNA sequence which is at least 96.13%, preferably at least 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to nucleotides 1-1401 of the DNA sequence of A. fumigatus ATCC 13073 alpha-mutant shown in Fig. 9.

A DNA sequence which encodes a phytase comprising an amino
20 acid which is at least 97.17%, preferably at least 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of A. fumigatus ATCC 13073 alpha-mutant shown in Fig. 9.

A DNA sequence which encodes a phytase and which (i) is at least 96.13%, preferably 96.5, 97, 97.5, 98, 98.5, 99, 99.5%
25 identical, or (ii) hybridizes under low, preferably medium, medium/high, high, very high stringency conditions to nucleotides 1-1401 of the DNA sequence of A. fumigatus ATCC 13073 alpha-mutant shown in Fig. 9. A preferred negative control is DNA encoding A. fumigatus 13073. A preferred positive control
30 is DNA encoding any of A. fumigatus ATCC 13073 and its optimised alpha-mutant.

Phytases and corresponding DNA sequences related to the optimized A. fumigatus alpha-mutant

A phytase which comprises an amino acid sequence which is
5 at least 96.08%, preferably at least 96.5, 97, 97.5, 98, 98.5,
99, 99.5% identical to the sequence of the phytase of the
optimized A. fumigatus alpha-mutant.

A phytase which comprises an amino acid sequence which is
at least 96.74%, preferably at least 97, 97.5, 98, 98.5, 99,
10 99.5% similar to the sequence of the phytase of the optimized A.
fumigatus alpha-mutant.

A phytase which is encoded by a DNA sequence which is at
least 95.63%, preferably at least 96, 96.5, 97, 97.5, 98, 98.5,
99, 99.5% identical to nucleotides 1-1401 of the DNA sequence
15 encoding the phytase of the optimized A. fumigatus alpha-mutant.

A DNA sequence which encodes a phytase comprising an amino
acid which is at least 96.08%, preferably at least 96.5, 97,
97.5, 98, 98.5, 99, 99.5% identical to the phytase of the
optimized A. fumigatus alpha-mutant.

20 A DNA sequence which encodes a phytase and which (i) is at
least 95.63%, preferably at least 96, 96.5, 97, 97.5, 98, 98.5,
99, 99.5% identical, or (ii) hybridizes under low, preferably
medium, medium/high, high, very high stringency conditions to
nucleotides 1-1401 of the DNA sequence encoding the phytase of
25 the optimized A. fumigatus alpha-mutant.

A preferred negative control is DNA encoding A. fumigatus
13073. A preferred positive control is DNA encoding any of A.
fumigatus ATCC 13073 and its optimised alpha-mutant.

30 Phytases and corresponding DNA sequences related to
consensus phytase 7

A phytase which comprises an amino acid sequence which is at least 94.87%, preferably at least 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 7 as shown in Fig. 10.

5 A phytase which comprises an amino acid sequence which is at least 95.30%, preferably at least 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-467 of consensus phytase 7 as shown in Fig. 10.

A phytase which is encoded by a DNA sequence which is at least 96.38%, preferably 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to nucleotides 12-1412 of the DNA sequence of consensus phytase 7 shown in Fig. 10.

A DNA sequence which encodes a phytase and which (i) is at least 96.38%, preferably at least 96.5, 97, 97.5, 98, 98.5, 99, 15 99.5% identical, or (ii) hybridizes under low, preferably medium, medium/high, high, very high stringency conditions to nucleotides 12-1412 of the DNA sequence of consensus phytase 7 as shown in Fig. 10.

A DNA sequence which encodes a phytase comprising an amino acid sequence which is at least 94.87%, preferably at least 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 7 as shown in Fig. 10.

25 Phytases related to basidio consensus

A phytase which comprises an amino acid sequence which is at least 76.23%, preferably at least 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the combined 30 sequence of (i) amino acids 1-441 of basidio consensus shown in

Fig. 3, and (ii) amino acids 1-26 shown in Fig. 5 (the sequence of (ii) to be added at the N-terminal of the sequence of (i)).

A phytase which comprises an amino acid sequence which is at least 79.50%, preferably at least 80, 81, 82, 83, 84, 85, 86, 5 87, 88, 89, 90, 91, 92, 93, 94, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-441 of basidio consensus as shown in Fig. 3.

Phytases related to consensus phytase 12

10 A phytase which comprises an amino acid sequence which is at least 70, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 12 as shown in Fig. 21.

15 A phytase which comprises an amino acid sequence which is at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-467 of consensus phytase 12 as shown in Fig. 21.

Table 8

Comparison of phytase amino acid sequences

Phytase	CP10	CP10-thermo[3]Q50-T-K91A	CP1-thermo[8]	CP1-thermo[8]-Q50T-K91A	CP11	CP7	Basidio	A. fumigatus a-mutant	A. fumigatus a-mutant (opt.)
Consensus phytase	95.08/93.79	94.65/93.36	98.50/98.29	98.07/97.86	92.06/90.70	95.29/94.86	69.42/62.16	85.59/82.58	84.73/81.72
A. niger NRRL3135	79.48/76.46	79.05/76.03	80.35/77.75	79.91/77.32	79.27/76.31	84.02/81.64	67.19/59.32	74.07/70.11	74.95/70/99
A. terreus 9-A1	76.04/72.11	75.82/71.90	76.47/72.33	76.25/72.11	76.51/73.02	75.76/71.18	65.39/58.02	69.67/64.84	69.45/64.84
A. terreus cbs	79.04/75.11	78.82/74.89	79.48/75.76	79.26/75.55	77.19/73.27	79.17/75.00	66.92/59.65	72.59/67.76	72.37/67.76
E. nidulans	78.70/74.35	78.26/73.91	79.78/75.87	79.35/75.44	80.56/76.62	76.96/73.04	67.20/58.13	72.39/67.83	72.11/67.54
A. fumigatus 13073	82.93/80.31	82.50/79.87	82.31/79.04	81.88/78.60	81.36/78.64	80.13/76.20	63.54/57.91	97.82/97.16	96.73/96.07
A. fumigatus 32239	81.30/77.39	80.87/76.96	81.09/77.61	80.65/77.17	79.95/76.08	79.13/75.22	63.61/54.97	90.22/86.52	89.57/85.87
T. thermophilus	77.83/73.84	77.38/73.39	78.67/74.89	78.22/74.44	78.47/74.76	76.51/73.15	61.54/54.36	72.01/66.82	72.69/67.49
M. thermophila	69.16/62.81	69.48/63.33	69.27/62.84	69.59/63.36	69.65/63.06	68.82/62.13	65.56/57.91	66.21/58.45	66.44/58.68
T. lanuginosus	73.52/66.70	73.06/66.44	71.92/64.61	71.46/64.16	74.21/68.86	69.50/62.62	67.20/57.41	68.91/61.02	69.61/61.72
P. lycki	64.92/59.10	64.91/59.37	64.46/58.09	64.46/58.36	65.03/59.84	63.13/56.50	77.75/73.07	64.08/57.11	62.47/55.91
A. pedlades	64.51/51.81	64.86/51.94	62.98/51.41	63.33/51.54	64.50/52.30	63.05/51.15	78.92/74.71	61.64/52.38	62.13/53.07
P. involutus 1	66.67/58.07	66.67/58.33	64.84/56.51	64.84/56.77	63.30/54.52	65.33/56.53	79.49/76.22	59.59/51.81	59.95/52.20
P. involutus 2	65.54/55.70	65.30/55.53	66.85/56.87	66.58/56.68	66.30/56.35	64.27/54.13	78.09/74.59	61.26/52.62	61.04/52.47

T. pubescens	65.46/57.22	65.72/57.47	62.89/55.67	63.14/55.93	65.03/57.65	63.28/56.51	78.34/75.12	64.08/57.11	62.30/55.24
CP10	-	99.57/99.57	96.57/95.50	96.15/95.08	95.02/94.56	91.01/89.29	70.22/62.28	85.13/82.76	85.99/83.62
CP10t[3]Q50TK91A	99.57/99.57	-	96.15/95.08	96.57/95.50	94.56/94.10	90.58/88.87	70.47/62.28	85.13/82.76	85.99/83.62
CPithermo[8]	96.57/95.50	96.15/95.08	-	99.57/99.57	93.42/92.29	94.43/93.79	68.40/60.74	84.52/81.94	85.38/82.80
CP1t[8]Q50TK91A	96.15/95.08	96.57/95.50	99.57/99.57	-	92.97/91.84	94.00/93.36	68.64/60.74	84.52/81.94	85.38/82.80
CP11	95.02/94.56	94.56/94.10	93.42/92.29	92.97/91.84	-	88.44/86.62	68.27/59.73	82.23/79.73	83.37/80.87
CP7	91.01/89.29	90.58/88.87	94.43/93.79	94.00/93.36	88.44/86.62	-	69.80/62.69	81.94/78.71	81.72/78.50
Basidio	70.22/62.28	70.47/62.28	68.40/60.74	68.64/60.74	68.27/59.73	69.80/62.69	-	65.97/60.52	66.41/60.68
A. fumigatus a-mut.	85.13/82.76	85.13/82.76	84.52/81.94	84.52/81.94	82.23/79.73	81.94/78.71	65.97/60.52	-	98.93/98.93
A. fum a-mut -opt.	85.99/83.62	85.99/83.62	85.38/82.80	85.38/82.80	83.37/80.87	81.72/78.50	66.41/60.68	98.93/98.93	-

Table 9

Comparison of phytase encoding DNA sequences

Phytase	CP10	CP10-thermo [3] Q50 T-K91A	CP1-thermo [8]	CP1-thermo [8] - Q50T-K91A	CP7	Basidio	A. fumigatus a-mutant	A. fumigatus a-mutant (opt.)
Consensus phytase	95.87	95.87	98.72	98.36	96.37	65.46	66.88	66.88
A. niger NRRL3135	65.10	64.82	66.10	65.74	67.52	50.68	65.88	66.17
A. terreus 9-Al	61.74	61.53	62.17	62.03	60.53	49.40	66.24	66.31
A. terreus cbs	62.52	62.30	63.02	62.88	61.45	49.74	68.17	68.24
E. nidulans	65.08	64.94	65.30	65.01	64.22	49.92	64.90	65.44
A. fumigatus 13073	65.66	65.38	64.19	64.08	63.65	48.27	96.12	95.62
T. thermophilus	62.52	62.50	62.53	62.66	62.00	52.19	61.77	61.92
M. thermophila	55.51	55.15	55.36	55.22	53.91	48.44	58.17	58.24
T. lanuginosus	57.56	57.20	56.76	56.47	62.00	44.66	59.71	60.07
P. lycii	45.76	46.51	45.14	55.21	55.46	58.50	48.91	49.44
A. pediatdes	49.89	49.89	49.89	50.11	45.54	61.66	47.49	47.56
P. involutus 1	48.32	49.03	47.81	47.96	49.59	59.80	49.96	50.19
P. involutus 2	48.24	49.00	48.08	48.63	47.94	60.16	47.56	47.63
T. pubescens	47.00	47.17	46.46	47.62	46.83	60.37	49.89	49.96
CP10	-	99.43	96.40	96.05	93.73	66.40	67.81	68.24

CP10C [3]Q50TK91A	99.43	-	96.37	96.58	93.45	66.29	67.81	68.24
Cpilthermo [8]	96.40	96.37	-	99.65	95.30	65.40	66.74	67.17
CP1t [8]Q50TK91A	96.05	96.58	99.65	-	94.94	65.47	66.74	67.17
CP7	93.73	93.45	95.30	94.94	-	64.56	65.88	65.88
Basidio	66.40	66.29	65.40	65.47	64.56	-	50.41	50.49
A. fumigatus a-mut.	67.81	67.81	66.74	66.74	65.88	50.41	-	99.50
A. fum a-mut -opt.	68.24	68.24	67.17	67.17	65.88	50.49	99.50	-

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SEQUENCE LISTING
to follow!

CLAIMS

1. A phytase which comprises an amino acid sequence which is at least 93.80% identical to the sequence of amino acids 1-467 of consensus phytase 10 (Fcpl0) as shown in Fig. 5.
2. A phytase which is encoded by a DNA sequence which is at least 95.88% identical to nucleotides 12-1412 of the DNA sequence of consensus phytase 10 (Fcpl0) as shown in Fig. 5.
3. A phytase which comprises an amino acid sequence selected from amongst the amino acid sequence of Fig. 5, and the amino acid sequence designated Fcpl0 shown in Fig. 4 (consensus phytase 10).
4. A phytase which comprises an amino acid sequence selected from amongst the amino acid sequences of (i) consensus phytase-10-thermo[3] (also designated consensus phytase-10-thermo); (ii) the variant Q50T, (iii) K91A, or (iv) (Q50T+K91A) thereof - variant (iv) is shown in Fig. 8; and (v) amino acids 27-467 of any of the sequences (i) to (iv).
5. A phytase which comprises an amino acid sequence selected from amongst the amino acid sequences of (i) consensus phytase-1-thermo[8] (also designated consensus phytase-1-thermo); (ii) the variant Q50T, (iii) K91A, or (iv) (Q50T+K91A) thereof - variant (iv) is shown in Fig. 7; and (v) amino acids 27-467 of any of the sequences of (i) to (iv).
6. A phytase which comprises the amino acid sequence of consensus phytase-11 shown in Fig. 6.

7. A DNA sequence which encodes the phytase of any one of claims 1-6.
- 5 8. A DNA sequence which encodes a phytase, and which is (i) at least 95.88% identical, or (ii) hybridizes under high stringency conditions, to nucleotides 12-1412 of the DNA sequence of consensus phytase 10 (Fcp10) as shown in Fig. 5.
- 10 9. A DNA sequence which encodes a phytase comprising an amino acid sequence which is at least 93.80% identical to the sequence of amino acids 1-467 of consensus phytase 10 (Fcp10) as shown in Fig. 5.
- 15 10. A DNA sequence which encodes a phytase, and which comprises
- (i) nucleotides 1-1426, 12-1412, 90-1426 or 90-1412 of the DNA sequence encoding consensus phytase 10 (Fcp 10) shown in Fig. 5;
 - (ii) nucleotides 1-1404, 1-1401, 79-1404, or 79-1401 of
 - 20 the DNA sequence encoding consensus phytase-10-thermo[3]-Q50T, K91A shown in Fig. 8;
 - (iii) variants of the nucleotides of (ii) which encodes variants (i)-(iii) of claim 4;
 - (iv) nucleotides 1-1410, 1-1407, 79-1410 or 79-1407 of
 - 25 the DNA sequence encoding consensus phytase-1-thermo[8]-Q50T, K91A as shown in Fig. 7;
 - (v) variants of the nucleotides of (iv) which encodes variants (i)-(iii) of claim 5; or
- 30 11. A vector comprising the DNA sequence according to any one of claims 3-5.

12. A host cell comprising the DNA sequence according to any one of claims 3-5 or the vector according to claim 6.
- 5 13. A process for producing a phytase, the process comprising culturing the host cell according to claim 7 under conditions permitting the production of the phytase, and recovering the phytase from the culture broth.
- 10 14. A food, feed or pharmaceutical composition comprising the phytase of any one of claims 1-2.

ABSTRACT

This invention relates to improved phytases, preferably phytases of an increased thermostability, and a process of producing them based on a comparison with consensus phytases. In particular, stabilizing amino acid mutations are introduced into a homologous protein, or the active site of a phytase is replaced in part or in toto. The corresponding DNA sequences and methods of preparing it is also disclosed, as are methods of producing the improved phytases, and the use thereof. Specific variants of *Aspergillus fumigatus* phytase and consensus phytases are disclosed.

Figure 1

	1		50
<i>A. terreus</i> 9A-1	KhsDCNSVDh	GYQCFPELSH	kwGLYAPYFS LQDESPPFLD VPEDChITFV
<i>A. terreus</i> cbs	NhsDCTSVDr	GYQCFPELSH	kwGLYAPYFS LQDESPPFLD VPDDChITFV
<i>A. niger</i> var. <i>awamori</i>	NqsTCDTVdQ	GYQCFSETSH	LGWQYAPFFS LANESAISPD VPAGCrVTFA
<i>A. niger</i> T213	NqsSCD TVDQ	GYQCFSETSH	LGWQYAPFFS LANESVISPD VPAGCrVTFA
<i>A. niger</i> NRRL3135	NqsSCD TVDQ	GYQCFSETSH	LGWQYAPFFS LANESVISPE VPAGCrVTFA
<i>A. fumigatus</i> 13073	GSkSCD TVDl	GYQCsPATSH	LGWQYSPFFS LEDElSVSSK LPKDCrITLV
<i>A. fumigatus</i> 32722	GSkSCD TVDl	GYQCsPATSH	LGWQYSPFFS LEDElSVSSK LPKDCrITLV
<i>A. fumigatus</i> 58128	GSkSCD TVDl	GYQCsPATSH	LGWQYSPFFS LEDElSVSSK LPKDCrITLV
<i>A. fumigatus</i> 26906	GSkSCD TVDl	GYQCsPATSH	LGWQYSPFFS LEDElSVSSK LPKDCrITLV
<i>A. fumigatus</i> 32239	GSkACD TVEl	GYQCsPGTSH	LGWQYSPFFS LEDElSVSSD LPKDCrVTFV
<i>E. nidulans</i>	QNHSCNTADG	GYQCFPNVSH	VWGQYSPYFS IEQESAISeD VPHGCeVTFV
<i>T. thermophilus</i>	DSHSCNTVEG	GYQCrPEISH	sWGQYSPFFS LADQSEISPD VPQNCrITFV
<i>M. thermophila</i>	ESRPCDTpDl	GFQCgTAISH	FWGQYSPYFS VpSElDaS.. IPDDCeVTFa
Consensus	NSHSCD TVDG	GYQCFPEISH	LGWQYSPYFS LEDESAISPD VPDDC-VTFV
Consensus phytase	NSHSCD TVDG	GYQCFPEISH	LGWQYSPYFS LEDESAISPD VPDDCrVTFV
	51		100
<i>A. terreus</i> 9A-1	QVLARHGARS	PTHSktKAYA	AtIAAIQKSA TaFpGKYAFL QSYNYSLDSE
<i>A. terreus</i> cbs	QVLARHGARS	PTDSKtKAYA	AtIAAIQKNA TaLpGKYAFL KSYNYSMGSE
<i>A. niger</i> var. <i>awamori</i>	QVLSRHGARY	PTESKgKkYS	ALIEEIQQNV TtFDGKYAFL KTYNYSLGAD
<i>A. niger</i> T213	QVLSRHGARY	PTESKgKkYS	ALIEEIQQNV TtFDGKYAFL KTYNYSLGAD
<i>A. niger</i> NRRL3135	QVLSRHGARY	PTDSKgKkYS	ALIEEIQQNA TtFDGKYAFL KTYNYSLGAD
<i>A. fumigatus</i> 13073	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA TdFKGKFAFL KTYNYTLGAD
<i>A. fumigatus</i> 32722	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA TdFKGKFAFL KTYNYTLGAD
<i>A. fumigatus</i> 58128	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA TdFKGKFAFL KTYNYTLGAD
<i>A. fumigatus</i> 26906	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA TdFKGKFAFL KTYNYTLGAD
<i>A. fumigatus</i> 32239	QVLSRHGARY	PTASKsKkYK	kLVTAIQKNA TeFKGKFAFL ETNYNYTLGAD
<i>E. nidulans</i>	QVLSRHGARY	PTESKsKAYS	GLIEAIQKNA TsFwCQYAFI ESYNYSNLGAD
<i>T. thermophilus</i>	QLLSRHGARY	PTSSKtElys	QLISrIQKTA TaYKGYAFL KDYrYqLGAN
<i>M. thermophila</i>	QVLSRHGARA	PTIKRaaSYv	DLIDrIHhGA IsYgPgYEFL RTYDYTLGAD
Consensus	QVLSRHGARY	PTSSK-KAYS	ALIEAIQKNA T-FKGKYAFL KTYNYTLGAD
Consensus phytase	QVLSRHGARY	PTSSKSKAYS	ALIEAIQKNA TAFKGKYAFL KTYNYTLGAD
	101		150
<i>A. terreus</i> 9A-1	ELTPFGrNQL	rDlGaQFYeR	YNALTrhInP FVRATDASRV hESAeKFVEG
<i>A. terreus</i> cbs	NLTPFGrNQL	qDlGaQFYRR	YDTLTrhInP FVRAADSSRV hESAeKFVEG
<i>A. niger</i> var. <i>awamori</i>	DLTPFGEQEL	VNSGIKFYQR	YESLTrNIIP FIRSSGSSRV IASGEKFIEG
<i>A. niger</i> T213	DLTPFGEQEL	VNSGIKFYQR	YESLTrNIIP FIRSSGSSRV IASGEKFIEG
<i>A. niger</i> NRRL3135	DLTPFGEQEL	VNSGIKFYQR	YESLTrNIIP FIRSSGSSRV IASGKKFIEG
<i>A. fumigatus</i> 13073	DLTPFGEQQL	VNSGIKFYQR	YKALARSVVP FIRASGSDRV IASGEKFIEG
<i>A. fumigatus</i> 32722	DLTPFGEQQL	VNSGIKFYQR	YKALARSVVP FIRASGSDRV IASGEKFIEG
<i>A. fumigatus</i> 58128	DLTPFGEQQL	VNSGIKFYQR	YKALARSVVP FIRASGSDRV IASGEKFIEG
<i>A. fumigatus</i> 26906	DLTAFGEQQL	VNSGIKFYQR	YKALARSVVP FIRASGSDRV IASGEKFIEG
<i>A. fumigatus</i> 32239	DLTPFGEQQM	VNSGIKFYQK	YKALAgSVVP FIRSSGSDRV IASGEKFIEG
<i>E. nidulans</i>	DLTiFGENQM	VDSGaKFYRR	YKNLARKnTP FIRASGSDRV VASAeKFIEG
<i>T. thermophilus</i>	DLTPFGENQM	IQlGIKFYnH	YKSLARNaVP FVRCSGSDRV IASGrIFIEG
<i>M. thermophila</i>	ELTRtGQQQM	VNSGIKFYRR	YRALARKsIP FVRTAGqDRV VhSAENFTQG
Consensus	DLTPFGENQM	VNSGIKFYRR	YKALARK-VP FVRASGSDRV IASAeKFIEG
Consensus phytase	DLTPFGENQM	VNSGIKFYRR	YKALARKIVP FIRASGSDRV IASAeKFIEG

	151		200
<i>A. terreus</i> 9A-1	FQTARqDDHh ANpHQPSPrV DVaIPEGSAY NNTLEHS1CT AFES...STV		
<i>A. terreus</i> cbs	FQNARqGDPH ANpHQPSPrV DVVIPEGTAY NNTLEHS1CT AFEA...STV		
<i>A. niger</i> var. <i>awamori</i>	FQSTKLkDPr AqpgQSSPkI DVVISEASSs NNTLDPGTCT VFED...SEL		
<i>A. niger</i> T213	FQSTKLkDPr AqpgQSSPkI DVVISEASSs NNTLDPGTCT VFED...SEL		
<i>A. niger</i> NRRL3135	FQSTKLkDPr AqpgQSSPkI DVVISEASSs NNTLDPGTCT VFED...SEL		
<i>A. fumigatus</i> 13073	FQqAKLADPG A.TNRAAPAI SVIIPSETF NNTLDHGVCT kFEA...SQL		
<i>A. fumigatus</i> 32722	FQqAKLADPG A.TNRAAPAI SVIIPSETF NNTLDHGVCT kFEA...SQL		
<i>A. fumigatus</i> 58128	FQqAKLADPG A.TNRAAPAI SVIIPSETF NNTLDHGVCT kFEA...SQL		
<i>A. fumigatus</i> 26906	FQqAKLADPG A.TNRAAPAI SVIIPSETF NNTLDHGVCT kFEA...SQL		
<i>A. fumigatus</i> 32239	FQqANVADPG A.TNRAAPVI SVIIPSETY NNTLDHVSCT NFEA...SEL		
<i>E. nidulans</i>	FRKAQLhdHG S..gQATPVV NVIIPEIDGF NNTLDHSTCV SFEN...DEr		
<i>T. thermophilus</i>	FQSAKVLDPh SDkHDAPPTI NVIIeEGPSY NNTLDtGSCP VFED...SSg		
<i>M. thermophila</i>	FHSALLADRG STvRPTlPyd mVVIPETAGa NNTLHNDlCT AFEEgpySTI		
Consensus	FQSAKLADPG S-PHQASPVl NVIIPEGSY NNTLDHGTCT AFED---SEL		
Consensus phytase	FQSAKLADPG SQPHQASPVl DVIIPEGSY NNTLDHGTCT AFED...SEL		
	201		250
<i>A. terreus</i> 9A-1	GDDAvANFTA VFAPAIaQRL EADLPgVqLS TDDVvNLMAM CPFETVSlTD		
<i>A. terreus</i> cbs	GDAADNFTA VFAPAIakRL EADLPgVqLS ADDVvNLMAM CPFETVSlTD		
<i>A. niger</i> var. <i>awamori</i>	ADTVEANFTA TFAPSIRQRL ENDLsgVTLT DTEVTyLMDM CSFDTIstST		
<i>A. niger</i> T213	ADTVEANFTA TFAPSIRQRL ENDLsgVTLT DTEVTyLMDM CSFDTIstST		
<i>A. niger</i> NRRL3135	ADTVEANFTA TFVPSIRQRL ENDLsgVTLT DTEVTyLMDM CSFDTIstST		
<i>A. fumigatus</i> 13073	GDEVAANFTA lFAPDIRARa EkHLPgVTLT DEDVVsLMDM CSFDTVARTS		
<i>A. fumigatus</i> 32722	GDEVAANFTA lFAPDIRARa EkHLPgVTLT DEDVVsLMDM CSFDTVARTS		
<i>A. fumigatus</i> 58128	GDEVAANFTA lFAPDIRARa EkHLPgVTLT DEDVVsLMDM CSFDTVARTS		
<i>A. fumigatus</i> 26906	GDEVAANFTA lFAPDIRARa KkHLPgVTLT DEDVVsLMDM CSFDTVARTS		
<i>A. fumigatus</i> 32239	GDEVEANFTA lFAPAIRARI EkHLPgVqLT DDDVVsLMDM CSFDTVARTS		
<i>E. nidulans</i>	ADEiEANFTA IMGPPIrkRL ENDLPGIKLT NENViyLMDM CSFDTMARTA		
<i>T. thermophilus</i>	GHDAQEKfak qFAPAIIEKI KDHLPGVDLA vSDVpyLMDL CPFETLARNh		
<i>M. thermophila</i>	GDDAQDTyLS TFAGPIcARV NANLPGANLT DADTVaLMDL CPFETVAaSS		
Consensus	GDDAEANFTA TFAPAIRARL EADLPgVTLT DEDVV-LMDM CPFETVARTS		
Consensus phytase	GDDVEANFTA LFAPAIRARL EADLPgVTLT DEDVVyLMDM CPFETVARTS		
	251		300
<i>A. terreus</i> 9A-1DAhTLSPFC DLFTAtEWtq YNYLlSLDKY YGYGGGNPLG		
<i>A. terreus</i> cbsDAhTLSPFC DLFTAAEWtq YNYLlSLDKY YGYGGGNPLG		
<i>A. niger</i> var. <i>awamori</i>vDTKLSPFC DLFTHdEWih YDYlQSLkKY YGHGAGNPLG		
<i>A. niger</i> T213vDTKLSPFC DLFTHdEWih YDYlQSLkKY YGHGAGNPLG		
<i>A. niger</i> NRRL3135vDTKLSPFC DLFTHdEWin YDYlQSLkKY YGHGAGNPLG		
<i>A. fumigatus</i> 13073DASQLSPFC QLFTHnEWkk YNYlQSLGKY YGYGAGNPLG		
<i>A. fumigatus</i> 32722DASQLSPFC QLFTHnEWkk YNYlQSLGKY YGYGAGNPLG		
<i>A. fumigatus</i> 58128DASQLSPFC QLFTHnEWkk YNYlQSLGKY YGYGAGNPLG		
<i>A. fumigatus</i> 26906DASQLSPFC QLFTHnEWkk YNYlQSLGKY YGYGAGNPLG		
<i>A. fumigatus</i> 32239DASELSPFC AIFTHnEWkk YDYlQSLGKY YGYGAGNPLG		
<i>E. nidulans</i>HGTELSPFC AIFTEkEWlq YDYlQSLSKY YGYGAGSPLG		
<i>T. thermophilus</i>TDT.LSPFC ALStQeEWqa YDYYQSLGKY YGnGGGNPLG		
<i>M. thermophila</i>	sdpataadagg gNGrpLSPFC rLFSEsEWra YDYlQSVGKW YGYGPGNPLG		
Consensus	----- -DATELSPFC ALFTE-EW-- YDYlQSLGKY YGYGAGNPLG		
Consensus phytaseDATELSPFC ALFTHDEWRQ YDYlQSLGKY YGYGAGNPLG		

	301				350
A. terreus 9A-1	PVQGVGWaNE	LMARLTRAPV	HDHTCVNNTL	DASPATFPLN	ATLYADFSHD
A. terreus cbs	PVQGVGWaNE	LIARLTRSPV	HDHTCVNNTL	DANPATFPLN	ATLYADFSHD
A. niger var. awamori	PTQGVGYaNE	LIARLTHSPV	HDDTSSNHTL	DSNPATFPLN	STLYADFSHD
A. niger T213	PTQGVGYaNE	LIARLTHSPV	HDDTSSNHTL	DSNPATFPLN	STLYADFSHD
A. niger NRRL3135	PTQGVGYaNE	LIARLTHSPV	HDDTSSNHTL	DSSPATFPLN	STLYADFSHD
A. fumigatus 13073	PAQGIGFtNE	LIARLTRSPV	QDHTSTNsTL	vSNPATFPLN	ATMYVDFSHD
A. fumigatus 32722	PAQGIGFtNE	LIARLTRSPV	QDHTSTNsTL	vSNPATFPLN	ATMYVDFSHD
A. fumigatus 58128	PAQGIGFtNE	LIARLTRSPV	QDHTSTNsTL	vSNPATFPLN	ATMYVDFSHD
A. fumigatus 26906	PAQGIGFtNE	LIARLTRSPV	QDHTSTNsTL	vSNPATFPLN	ATMYVDFSHD
A. fumigatus 32239	PAQGIGFtNE	LIARLTNSPV	QDHTSTNsTL	DSDPATFPLN	ATIVVDFSHD
E. nidulans	PAQGIGFtNE	LIARLTQSPV	QDNTSTNHTL	DSNPATFPLD	rKLYADFSHD
T. thermophilus	PAQGVGFvNE	LIARMTHSPV	QDYTTVNHTL	DSNPATFPLN	ATLYADFSHD
M. thermophila	PTQGVGFvNE	LLARLAGvPV	RDgTSTNRTL	DGDPrTFPLG	rPLYADFSHD
Consensus	PAQGVGF-NE	LIARLTHSPV	QDHTSTNHTL	DSNPATFPLN	ATLYADFSHD
Consensus phytase	PAQGVGFANE	LIARLTRSPV	QDHTSTNHTL	DSNPATFPLN	ATLYADFSHD
	351				400
A. terreus 9A-1	SNLVSIFWAL	GLYNGTAPLS	qTSVESVSQT	DGYAAAWTVP	FAARAYVEMM
A. terreus cbs	SNLVSIFWAL	GLYNGTkPLS	qTTVEDITrT	DGYAAAWTVP	FAARAYIEMM
A. niger var. awamori	NGIISILFAL	GLYNGTkPLS	TTTVENITQT	DGFSSAWTVP	FASRLYVEMM
A. niger T213	NGIISILFAL	GLYNGTkPLS	TTTVENITQT	DGFSSAWTVP	FASRLYVEMM
A. niger NRRL3135	NGIISILFAL	GLYNGTkPLS	TTTVENITQT	DGFSSAWTVP	FASRLYVEMM
A. fumigatus 13073	NSMVSIFFAL	GLYNGTEPLS	rTSVESaKEl	DGYSASWVVP	FGARAYFetM
A. fumigatus 32722	NSMVSIFFAL	GLYNGTGPLS	rTSVESaKEl	DGYSASWVVP	FGARAYFetM
A. fumigatus 58128	NSMVSIFFAL	GLYNGTEPLS	rTSVESaKEl	DGYSASWVVP	FGARAYFetM
A. fumigatus 26906	NSMVSIFFAL	GLYNGTEPLS	rTSVESaKEl	DGYSASWVVP	FGARAYFetM
A. fumigatus 32239	NGMIPIFFAM	GLYNGTEPLS	qTSeESTKES	NGYSASWAVP	FGARAYFetM
E. nidulans	NSMISIFFAM	GLYNGTQPLS	mDSVESIQEm	DGYAASWTVP	FGARAYFELM
T. thermophilus	NTMTSIFaAL	GLYNGTAKLS	TTEIKSIEET	DGYSAAWTVP	FGGRAYIEMM
M. thermophila	NDMMGVLgAL	GaYDGVPLD	KTArrDpEEl	GGYAASWAVP	FAARIYVEKM
Consensus	NSMISIFFAL	GLYNGTAPLS	TTSVESIEET	DGYAASWTVP	FGARAYVEMM
Consensus phytase	NSMISIFFAL	GLYNGTAPLS	TTSVESIEET	DGYSASWTVP	FGARAYVEMM
	401				450
A. terreus 9A-1	QC.....RAEKE	PLVRVLVNDR	VMPLHGCPD	KLGRCKrDAF
A. terreus cbs	QC.....RAEKQ	PLVRVLVNDR	VMPLHGCAVD	NLGRCKrDDF
A. niger var. awamori	QC.....QAEQE	PLVRVLVNDR	VVPLHGCPID	aLGRCTrDSF
A. niger T213	QC.....QAEQE	PLVRVLVNDR	VVPLHGCPID	aLGRCTrDSF
A. niger NRRL3135	QC.....QAEQE	PLVRVLVNDR	VVPLHGCPVD	aLGRCTrDSF
A. fumigatus 13073	QC.....KSEKE	PLVRALINDR	VVPLHGCDVD	KLGRCKLNDf
A. fumigatus 32722	QC.....KSEKE	PLVRALINDR	VVPLHGCDVD	KLGRCKLNDf
A. fumigatus 58128	QC.....KSEKE	SLVRALINDR	VVPLHGCDVD	KLGRCKLNDf
A. fumigatus 26906	QC.....KSEKE	PLVRALINDR	VVPLHGCDVD	KLGRCKLNDf
A. fumigatus 32239	QC.....KSEKE	PLVRALINDR	VVPLHGCAVD	KLGRCKLKDF
E. nidulans	QC.....E.KKE	PLVRVLVNDR	VVPLHGCAVD	KFGRCTLDdW
T. thermophilus	QC.....DDSD	PVVRVLVNDR	VVPLHGCEVD	SLGRCKrDDF
M. thermophila	RCsggggggg	gggrQEKE	eMVRVLVNDR	VMTLkGCGAD	ErGMCTLErF
Consensus	QC-----	-----QAEKE	PLVRVLVNDR	VVPLHGCAVD	KLGRCKLDDF
Consensus phytase	QC.....QAEKE	PLVRVLVNDR	VVPLHGCAVD	KLGRCKRDDF

	451	471
<i>A. terreus</i> 9A-1	VAGLSFAQAG	GNWADCF--- -
<i>A. terreus</i> cbs	VEGLSFARAG	NWAECF---
<i>A. niger</i> var. <i>awamori</i>	VrGLSFARSG	GDWAECsA-- -
<i>A. niger</i> T213	VrGLSFARSG	GDWAECFA-- -
<i>A. niger</i> NRRL3135	VrGLSFARSG	DWAECFA~~
<i>A. fumigatus</i> 13073	VKGLSWARSG	GNWGECS-- -
<i>A. fumigatus</i> 32722	VKGLSWARSG	GNWGECS-- -
<i>A. fumigatus</i> 58128	VKGLSWARSG	GNWGECS-- -
<i>A. fumigatus</i> 26906	VKGLSWARSG	GNWGECS-- -
<i>A. fumigatus</i> 32239	VKGLSWARSG	NSEQSFS~~
<i>E. nidulans</i>	VEGLNFARSG	GNWkTCFT1- -
<i>T. thermophilus</i>	VrGLSFARqG	GNWEGCYAas e
<i>M. thermophila</i>	IESMAFARGN	GKWD1CFA-- -
Consensus	VEGLSFARSG	GNWAECFA-- -
Consensus phytase	VEGLSFARSG	GNWAECFA... .

Figure 2

CP-1
 Eco RI M G V F V V L L S I A T L F G S T
 TATATGAATTCATGGGCGTGTTCGTCTGCTACTGTCCATTGCCACCTTGTTCGGTTCCA
 1 -----+-----+-----+-----+-----+-----+ 60
 ATATACTTAAGTACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAAACAAGCCAAGGT

 S G T A L G P R G N S H S C D T V D G G
 CATCCGGTACCGCCTTGGGTCCTCGTGTAATTCTCACTCTTGTGACACTGTTGACGGTG
 61 -----+-----+-----+-----+-----+-----+ 120
 GTAGGCCATGGCGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCAC
 CP-2
 CP-3
 Y Q C F P E I S H L W G Q Y S P Y F S L
 GTTACCAATGTTTCCAGAAATTTCTCACTTGTGGGGTCAATACTCTCCATACTTCTCTT
 121 -----+-----+-----+-----+-----+-----+ 180
 CAATGGTTACAAAGGGTCTTTAAAGAGTGAACACCCAGTTATGAGAGGTATGAAGAGAA

 E D E S A I S P D V P D D C R V T F V Q
 TGAAGACGAATCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTTCGTTT
 181 -----+-----+-----+-----+-----+-----+ 240
 ACCTTCTGCTTAGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAG
 CP-4
 CP-5
 V L S R H G A R Y P T S S K S K A Y S A
 AAGTTTTGTCTAGACACGGTGCTAGATACCCAACTTCTTCTAAGTCTAAGGCTTACTCTG
 241 -----+-----+-----+-----+-----+-----+ 300
 TTCAAACAGATCTGTGCCACGATCTATGGGTTGAAGAAGATTGAGATTCCGAATGAGAC

 L I E A I Q K N A T A F K G K Y A F L K
 CTTTGATTGAAGCTATTCAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGA
 301 -----+-----+-----+-----+-----+-----+ 360
 GAAACTAACTTCGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAAGT
 CP-6
 CP-7
 T Y N Y T L G A D D L T P F G E N Q M V
 AGACTTACAACCTACACTTTGGGTGCTGACGACTTGACTCCATTGCGTGAAAACCAAATGG
 361 -----+-----+-----+-----+-----+-----+ 420
 TCTGAATGTTGATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACC

 N S G I K F Y R R Y K A L A R K I V P F
 TTAAGTCTGGTATTAAGTTCTACAGAAGATAACAAGGCTTTGGCTAGAAAGATTGTTCCAT
 421 -----+-----+-----+-----+-----+-----+ 480
 AATTGAGACCATAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTA
 CP-8
 CP-9
 I R A S G S D R V I A S A E K F I E G F
 TCATTAGAGCTTCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTTCAATTGAAGGTT
 481 -----+-----+-----+-----+-----+-----+ 540
 AGTAATCTCGAAGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAAGTTCCAA

 Q S A K L A D P G S Q P H Q A S P V I D
 TCCAATCTGCTAAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTG
 541 -----+-----+-----+-----+-----+-----+ 600
 AGGTTAGACGATTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTTGAAGAGGTCAATAAC

CP-10

CP-11

V I I P E G S G Y N N T L D H G T C T A
 ACGTTATTATTCCAGAAGGaTCcGGTTACAACAACACTTTGGACCACGGTACTTGTACTG
 601 -----+-----+-----+-----+-----+ 660
 TGCAATAATAAGGTCTTCctAGgCCAATGTTGTTGTGAAACCTGGTGCCATGAACATGAC

F E D S E L G D D V E A N F T A L F A P
 CTTTCGAAGACTCTGAATTGGGTGACGACGTTGAAGCTAACTTCACTGCTTTGTTGCTC
 661 -----+-----+-----+-----+-----+ 720
 GAAAGCTTCTGAGACTTAACCCACTGCTGCAACTTCGATTGAAGTGACGAAACAAGCGAG

CP-12

A I R A R L E A D L P G V T L T D E D V
 CAGCTATTAGAGCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACGAAGACG
 721 -----+-----+-----+-----+-----+ 780
 GTCGATAATCTCGATCTAACCTTCGACTGAACGGTCCACAATGAACTGACTGCTTCTGC

CP-13

V Y L M D M C P F E T V A R T S D A T E
 TTGTTTACTTGATGGACATGTGTCCATTTCGAACTGTTGCTAGAACTTCTGACGCTACTG
 781 -----+-----+-----+-----+-----+ 840
 AACAAATGAACTACCTGTACACAGGTAAGCTTTGACAACGATCTTGAAGACTGCGATGAC

L S P F C A L F T H D E W R Q Y D Y L Q
 AATTGTCTCCATTCTGTGCTTTGTTCACTCACGACGAATGGAGACAATACGACTACTTGC
 841 -----+-----+-----+-----+-----+ 900
 TTAACAGAGGTAAGACACGAAACAAGTGAGTGCTGCTTACCTCTGTTATGCTGATGAACG

CP-14

CP-15

S L G K Y Y G Y G A G N P L G P A Q G V
 AATCTTTGGGTAAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTG
 901 -----+-----+-----+-----+-----+ 960
 TTAGAAACCCATTGATGATGCCAATGCCACGACCATTGGGTAACCCAGGTGAGTTCCAC

G F A N E L I A R L T R S P V Q D H T S
 TTGGTTTCGCTAACGAATTGATTGCTAGATTGACTAGATCTCCAGTTCAAGACCACACT
 961 -----+-----+-----+-----+-----+ 1020
 AACCAAAGCGATTGCTTAACCTAACGATCTAACTGATCTAGAGGTCAAGTTCTGGTGTGAA

CP-16

CP-17

T N H T L D S N P A T F P L N A T L Y A
 CTACTAACCACACTTTGGACTCTAACCCAGCTACTTTCCCATTTGAACGCTACTTTGTACG
 1021 -----+-----+-----+-----+-----+ 1080
 GATGATTGGTGTGAAACCTGAGATTGGGTCGATGAAAGGGTAACCTTGCATGAAACATGC

D F S H D N S M I S I F F A L G L Y N G
 CTGACTTCTCTCACGACAACTCTATGATTTCTATTTCTTCGCTTTGGGTTTGTACAACG
 1081 -----+-----+-----+-----+-----+ 1140
 GACTGAAGAGAGTGCTGTTGAGATACTAAAGATAAAAGAAGCGAAACCCAAACATGTTGC

CP-18

CP-19

T A P L S T T S V E S I E E T D G Y S A
 GTACTGCTCCATTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTTACTCTG
 1141 -----+-----+-----+-----+-----+ 1200
 CATGACGAGGTAACAGATGATGAAGACAACCTTAGATAACTTCTTTGACTGCCAATGAGAC

S W T V P F G A R A Y V E M M Q C Q A E
 CTTCTTGGACTGTTCCATTTCGGTGCTAGAGCTTACGTTGAAATGATGCAATGTCAAGCTG
 1201 -----+-----+-----+-----+-----+ 1260
 GAAGAACCTGACAAGGTAAGCCACGATCTCGAATGCAACTTTACTACGTTACAGTTTCGAC

CP-20

K E P L V R V L V N D R V V P L H G C A
 AAAAGGAACCATTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTG
 1261 -----+-----+-----+-----+-----+ 1320
 TTTTCCTTGGTAACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACAC

CP-21

R V D K L G R C K R D D F V E G L S F A

CTGTTGACAAGTTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTTCGCTA
 1321 -----+-----+-----+-----+-----+ 1380
 GACAACTGTTCAACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGAT

CP-22

S G G N W A E C F A * Eco RI
 GATCTGGTGGTAACTGGGCTGAATGTTTCGCTTAAGAATTCATATA
 1381 -----+-----+-----+-----+----- 1426
 CTAGACCACCATTGACCCGACTTACAAAGCGAATTCTTAAGTATAT

Figure 3

	1		50
<i>P. involutus</i> (phyA1)	SvP.KnTAPt	FPIPeseQrn	WSPYSPYFPL AeYkAPPAGC QInQVNIIQR
<i>P. involutus</i> (phyA2)	SvP.RniAPK	FSIPeseQrn	WSPYSPYFPL AeYkAPPAGC EInQVNIIQR
<i>T. pubescens</i>	hiPlRdTSAc	LdVTrDvQqs	WSmYSPYFPa AtYvAPPASC QInQVHIIQR
<i>A. pediades</i>	GgvvQaTfvQ	pfFPPQiQds	WAAyTPYYPV qaYtPPPkDC KITQVNIIQR
<i>P. lycii</i>	StQsfvAAQ	LPIPaQntsn	WGPYdPFFPV EpYaAPPEGC tVtQVNLIQR
Basidio	S-P-R-TAAQ	LPIP-Q-Q--	WSPYSPYFPV A-Y-APPAGC QI-QVNIIQR
	51		100
<i>P. involutus</i> (phyA1)	HGARFPTSGA	TTRIKAGLTK	LQGvqnftDA KFNFIksfky dLGnsDLVFP
<i>P. involutus</i> (phyA2)	HGARFPTSGA	ATRIKAGLSK	LQSVqnftDP KFDFIkSfTY dLGtsDLVFP
<i>T. pubescens</i>	HGARFPTSGA	AkRIQTAVAK	LKAAsnyTDP lLAFVtNyTY sLGQdsLveL
<i>A. pediades</i>	HGARFPTSGA	GTRIQAaVKK	LQSAktyTDP RLDFLTnyTY tLGhDDLVPF
<i>P. lycii</i>	HGARWPTSGA	rSRqvaAVAK	IQmArpfTDP KYEFLnDfvy kFGvADLLPF
Basidio	HGARFPTSGA	ATRIQAaVAK	LQSA---TDP KLDFL-N-TY -LG-DDLVFP
	101		150
<i>P. involutus</i> (phyA1)	GAAQSfDAGQ	EAFARYSkLV	SkNNLPFIRA dGSDRVVDSA TNWTAGFAsA
<i>P. involutus</i> (phyA2)	GAAQSfDAGl	EvFARYSkLV	SsDNLPPFIRS dGSDRVVDTA TNWTAGFAsA
<i>T. pubescens</i>	GATQSSEAGQ	EAFTRYSSLV	SaDELPPFVRA SGSDRVVATA nNWTAGFAlA
<i>A. pediades</i>	GAlQSSQAGE	ETFqRYSfLV	SkENLPPFVRA SSSNRVVDSA TNWTEGFSaA
<i>P. lycii</i>	GAnQShQTGt	DmYTRYStLf	egGDVPFVRA AGDQRVVDSs TNWTAGFGdA
Basidio	GA-QSSQAGQ	EAFTRYs-LV	S-DNLPPFVRA SGSDRVVDSA TNWTAGFA-A
	151		200
<i>P. involutus</i> (phyA1)	ShNTvqPkLn	LILPQtGNDT	LEDNMCPaAG DSDPQvNaWL AVafPSITAR
<i>P. involutus</i> (phyA2)	SrNAiqPkLd	LILPQtGNDT	LEDNMCPaAG ESDPQvDaWL AsafPSVTAQ
<i>T. pubescens</i>	SsNSitPvLs	VIIEEaGNDT	LDDNMCPaAG DSDPQvNqWL AqFAPPMTAR
<i>A. pediades</i>	ShHvlnPiLf	VILSEsLNDT	LDDaMCPnAG sSDPQtGiWt SIYGTPIAnR
<i>P. lycii</i>	SgETvlPtLq	VVLqEeGNcT	LcNNMCPnEv DGDest.tWL GVFAPnITAR
Basidio	S-NT--P-L-	VILSE-GNDT	LDDNMCP-AG DSDPQ-N-WL AVFAPPITAR
	201		250
<i>P. involutus</i> (phyA1)	LNAAAPSvNL	TDtDAfNLvs	LCAFlTVSke kkSdFCtLFE giPGsFeAFa
<i>P. involutus</i> (phyA2)	LNAAAPGANL	TDaDAfNLvs	LCPFmTVSke qkSdFCtLFE giPGsFeAFa
<i>T. pubescens</i>	LNAGAPGANL	TDtDTyNLlt	LCPFETVatE rrSeFCDIYE elQAE.dAFa
<i>A. pediades</i>	LNqqAPGANI	TAaDvsNLip	LCAFETivke tpSpFCNLf. .tPEEFaqFe
<i>P. lycii</i>	LNAAAPSANL	SDsDAltLmd	MCPFDTLsG naSpFCDLf. .tAEEYvSYe
Basidio	LNAAAPGANL	TD-DA-NL--	LCPFETVS-E --S-FCDLFE --PEEF-AF-
	251		300
<i>P. involutus</i> (phyA1)	YgGDLDKfYG	TGYGQeLGPV	QGVGYVNELI ARLTnsAVRD NTQTNRtLDA
<i>P. involutus</i> (phyA2)	YaGDLDKfYG	TGYGQALGPV	QGVGYINELL ARLTnsAVnd NTQTNRtLDA
<i>T. pubescens</i>	YnADLDKfYG	TGYGQPLGPV	QGVGYINELI ARLTaQnVsD HTQTnsTLDS
<i>A. pediades</i>	YfGDLDKfYG	TGYGQPLGPV	QGVGYINELL ARLTemPVRD NTQTNRtLDS

P. lycii YyyDLdkYYG TGpGNALGPV QGVGYVNELL ARLTgQAVRD ETQTNRtLDS

Basidio Y-GDLdkFYG TGYGQPLGPV QGVGYINELL ARLT-QAVRD NTQTNRtLDS

	301		350
<i>P. involutus</i> (phyA1)	SPvTFPLNKT	FYADFShDNl	MVAVFSAMGL FrQPAPLsTS vPNPwRTWrT
<i>P. involutus</i> (phyA2)	APdTFPLNKT	MYADFShDNl	MVAVFSAMGL FrQSAPLsTS tPDPNRTWLT
<i>T. pubescens</i>	SPeTFPLNRT	LYADFShDNQ	MVAIFSAMGL FNQSAPLDPT tPDPaRTFLv
<i>A. pediades</i>	SPlTFPLDRS	IYADLShDNQ	MIAIFSAMGL FNQSSPLDPS fPNPKRTWVT
<i>P. lycii</i>	dPaTFPLNRT	FYADFShDNt	MVPIFAALGL FNaTA.LDPl kPDeNRlWVd

Basidio SP-TFPLNRT FYADFShDNQ MVAIFSAMGL FNQSAPLDPS -PDPNRTWVT

	351		400
<i>P. involutus</i> (phyA1)	SsLVPFSGRM	VVERLsC..f	GT.....tkv RVLVQDqVQP
<i>P. involutus</i> (phyA2)	SsVVPFSARM	aVERLsC..a	GT.....tkv RVLVQDqVQP
<i>T. pubescens</i>	kKIVPFsARM	VVERLdC..g	GA.....qsV RLLVNDAVQP
<i>A. pediades</i>	SRLtPFsARM	VtERLlCqrd	GTgsgggsri mrngnvqtfv RILVNDAVQP
<i>P. lycii</i>	SKLVPFSGHM	tVEKLsC...sgkeav RVLVNDAVQP

Basidio SKLVPFsARM VVERL-C--- GT-----V RVLVNDAVQP

	401		441
<i>P. involutus</i> (phyA1)	LEFCGGDrNG lCTLakFVES	QtFARsDGaG	DFEKCFATSa ~
<i>P. involutus</i> (phyA2)	LEFCGGDqDG lCALDkFVES	QaYARsGGaG	DFEKCLATTv ~
<i>T. pubescens</i>	LAFCGADtsG vCTLDAFVES	QaYARNDGEG	DFEKCFAT-- ~
<i>A. pediades</i>	LKFCGGDmDS lCTLEAFVES	QkYAREDGQG	DFEKCFD--- ~
<i>P. lycii</i>	LEFCGG.vDG vCeLsAFVES	QtYARENGQG	DFAKCgfvPs e
Basidio	LEFCGGD-DG -CTLDAFVES	Q-YAREDGQG	DFEKCFATP- -

Figure 4

	1		50
<i>A. terreus</i> 9a1	KhSDCNSVDh	GYQCfPELSH	kWGLYAPYFS LqDESPFFlD VPEDCHITFV
<i>A. terreus</i> cbs	NhsdCtSVDr	GYQCfPELSH	kWGLYAPYFS LqDESPFFlD VPdDCHITFV
<i>A. niger</i> var. <i>awamori</i>	NqSTCDTVdQ	GYQCfSEtSH	LWGQYAPFFS LANESAISPD VPAGCRVTfA
<i>A. niger</i> NRRL3135	NqSsCDTVdQ	GYQCfSEtSH	LWGQYAPFFS LANESvISPE VPAGCRVTfA
<i>A. fumigatus</i> 13073	GskSCDTVDl	GYQCsPatSH	LWGQYSPFFS LEDElSVSSK LPkDCRITLV
<i>A. fumigatus</i> 32722	GskSCDTVDl	GYQCsPatSH	LWGQYSPFFS LEDElSVSSK LPkDCRITLV
<i>A. fumigatus</i> 58128	GskSCDTVDl	GYQCsPatSH	LWGQYSPFFS LEDElSVSSK LPkDCRITLV
<i>A. fumigatus</i> 26906	GskSCDTVDl	GYQCsPatSH	LWGQYSPFFS LEDElSVSSK LPkDCRITLV
<i>A. fumigatus</i> 32239	GskACDTVEl	GYQCsPGtSH	LWGQYSPFFS LEDElSVSSD LPkDCRVTFV
<i>E. nidulans</i>	QNHSCNTAdG	GYQCfPNVSH	VWGQYSPYFS IEQESAISeD VPhGCEvTFV
<i>T. thermophilus</i>	DSHSCNTVEG	GYQCrPEISH	sWGQYSPFFS LADQSEISPD VPqNCKITFV
<i>T. lanuginosa</i>	-----	-----nvDIAR	hWGQYSPFFS LAEvSEISPA VPkGCRVeFv
<i>M. thermophila</i>	ESRPCDTpDl	GFQCgTAISH	FWGQYSPYFS VPSElDaS.. IPdDCeVTFa
Basidio	xSxPxrxTAA	qLPipxQxqx	xWSPYSPYFP VAXyxA.... pPaGCQIXqV

Consensus NSHSCDTVDG GYQC-PEISH LWGQYSPFFS LADESAISPD VP-GCRVTFV
 Fcp10 NSHSCDTVDG GYQCfPEISH LWGQYSPFFS LADESAISPD VPkGCRVTFV

	51		100
<i>A. terreus</i> 9a1	QVLARHGArS	PThSKTKaYA	AtIaAIQKSA TaFpGKYAFL QSYNYSLDSE
<i>A. terreus</i> cbs	QVLARHGArS	PTdSKTKaYA	AtIaAIQKNA TaLpGKYAFL KSYNYSMGSE
<i>A. niger</i> var. <i>awamori</i>	QVLSRHGARY	PTeSKGKKYS	ALIEEIQQNv TtFDGKYAFL KTYNYSLGAD
<i>A. niger</i> NRRL3135	QVLSRHGARY	PTdSKGKKYS	ALIEEIQQNA TtFDGKYAFL KTYNYSLGAD
<i>A. fumigatus</i> 13073	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA TdFKGKFAFL KTYNYTLGAD
<i>A. fumigatus</i> 32722	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA TdFKGKFAFL KTYNYTLGAD
<i>A. fumigatus</i> 58128	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA TdFKGKFAFL KTYNYTLGAD
<i>A. fumigatus</i> 26906	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA TdFKGKFAFL KTYNYTLGAD
<i>A. fumigatus</i> 32239	QVLSRHGARY	PTASKSKKYk	kLVtAIQKNA TeFKGKFAFL ETYNYTLGAD
<i>E. nidulans</i>	QVLSRHGARY	PTeSKSKaYS	GLIEAIQKNA TsFwGQYAFI ESYNYTLGAD
<i>T. thermophilus</i>	QLLSRHGARY	PTSSKTELYS	qLIsrIQKtA TaYKGyYAFI KdYrYqLGAN
<i>T. lanuginosa</i>	QVLSRHGARY	PTAHKSEvYA	ELLqrIQDtA TeFKGDFAFL RdYaYhLGAD
<i>M. thermophila</i>	QVLSRHGARA	PTIkRAasYv	DLIdrIHhGA IsYgPgYEFL RTYDYTLGAD
Basidio	NIIqRHGARF	PTSGaAtRiq	AaVakLQsax xxtDPKLDPL xnxtYxLGxD

Consensus QVLSRHGARY PTSSKSKKYS ALI-AIQKNA T-FKGKYAFL KTYNYTLGAD
 Fcp10 QVLSRHGARY PTSSKSKKYS ALIEAIQKNA TAFKGKYAFL KTYNYTLGAD

	101		150
<i>A. terreus</i> 9a1	ELTPFGGrNQL	rDlGaQFYeR	YNAL.TRhIn PFVRATDAsR VhESAeKFVE
<i>A. terreus</i> cbs	NLTPFGGrNQL	qDlGaQFYRR	YDTL.TRhIn PFVRAADsSR VhESAeKFVE
<i>A. niger</i> var. <i>awamori</i>	DLTPFGEQEL	VNSGIKFYQR	YESL.TRnII PFIRSSGSsR VIASGEKFIE
<i>A. niger</i> NRRL3135	DLTPFGEQEL	VNSGIKFYQR	YESL.TRnIV PFIRSSGSsR VIASGEKFIE
<i>A. fumigatus</i> 13073	DLTPFGEQQL	VNSGIKFYQR	YKAL.AReVV PFIRASGSdR VIASGEKFIE
<i>A. fumigatus</i> 32722	DLTPFGEQQL	VNSGIKFYQR	YKAL.AReVV PFIRASGSdR VIASGEKFIE
<i>A. fumigatus</i> 58128	DLTPFGEQQL	VNSGIKFYQR	YKAL.AReVV PFIRASGSdR VIASGEKFIE
<i>A. fumigatus</i> 26906	DLTAFGEQQL	VNSGIKFYQR	YKAL.AReVV PFIRASGSdR VIASGEKFIE
<i>A. fumigatus</i> 32239	DLTPFGEQQM	VNSGIKFYQK	YKAL.AgsVV PFIRSSGSdR VIASGEKFIE
<i>E. nidulans</i>	DLTiFGENQM	VDSGaKFYRR	YKnL.Arknt PFIRASGSdR VVASAEKFIN
<i>T. thermophilus</i>	DLTPFGENQM	IQLGIKFYnH	YKSL.ARnaV PFVRCGSdR VIASGr1FIE
<i>T. lanuginosa</i>	NLTRFGEEQM	MESGrQFYHR	YREq.AReIV PFVRAAGSAR VIASAEfFnr
<i>M. thermophila</i>	ELTRtGOQQM	VNSGIKFYRR	YRAL.ARksI PFVRTAGqDR VVhSAENftQ
Basidio	DLvPFGAxQs	sQAGQeAFtR	YsxLvSxdnL PFVRASGSdR VVDSAtNwtA

Consensus DLTPFGEQQM VNSGIKFYRR YKAL-AR-IV PFVRASGSdR VIASAEKFIE
 Fcp10 DLTPFGEQQM VNSGIKFYRR YKAL.ARkIV PFVRASGSdR VIASAEKFIE

	151		200
A. terreus 9a1	GFQTARqDDh	hAnphQSPPr	VDVaIPEGsA YNNTLEHSLC TAFES...St
A. terreus cbs	GFQNARqGDP	hAnphQSPPr	VDVVIPEGtA YNNTLEHSIC TAFEa...St
A. niger var. awamori	GFQSTKLkDP	rAqpgQSSPk	IDVVISeAsS sNNTLDpGtC TvFEd...SE
A. niger NRRL3135	GFQSTKLkDP	rAqpgQSSPk	IDVVISeAsS sNNTLDpGtC TvFEd...SE
A. fumigatus 13073	GFQqAKLADP	gAt.nRAAPa	ISVIIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 32722	GFQqAKLADP	gAt.nRAAPa	ISVIIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 58128	GFQqAKLADP	gAt.nRAAPa	ISVIIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 26906	GFQqAKLADP	gAt.nRAAPa	ISVIIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 32239	GFQqANVADP	gAt.nRAAPV	ISVIIPESeT YNNTLDHSVC TnFEa...SE
E. nidulans	GFRkaQLhDh	g.s.gQATPV	VNVIIPeIdG FNNTLDHStC vSFEn...dE
T. thermophilus	GFQSAKVlDP	hSdKhDAPt	INVIIeEGpS YNNTLDtGsC PvFEd...Se
T. lanuginosa	GFQdAKdrDP	rSnkdQaEPV	INVIISeETG sNNTLDgltC PAAEe...Ap
M. thermophila	GFHSAALLADR	gStvrPTlPy	dmVVIPETaG aNNTLHNDLC TAFEegPySt
Basidio	GFaxA.....	..sxntxxPx	LxVILSExg. .NDTLDDNMCPxAG

Consensus	GFQSAKLADP	-A---QASPV	INVIIPEG-G	YNNTLDHGVC	TAFE--P-SE
Fcp10	GFQSAKLADP	GANPQASPV	INVIIPEGAG	YNNTLDHGVC	TAFEE...SE

	201		250
A. terreus 9a1	VGDDaVANFT	AVFAPAIaqr	LEAdLPGVQL StDDVVNLMA MCPFETVSlT
A. terreus cbs	VGDAaADNFT	AVFAPAIaqr	LEAdLPGVQL SADDVVNLMA MCPFETVSlT
A. niger var. awamori	LADtVEANFT	AtFAPSIRqR	LEndLSGvTL TdEVtyLMD MCSFDtIstS
A. niger NRRL3135	LADtVEANFT	AtFAPSIRqR	LEndLSGvTL TdEVtyLMD MCSFDtIstS
A. fumigatus 13073	LGDEVAANFT	ALFAPdIRAR	aEkhlPGVtL TDEDVVS LMD MCSFDtVArT
A. fumigatus 32722	LGDEVAANFT	ALFAPdIRAR	aEkhlPGVtL TDEDVVS LMD MCSFDtVArT
A. fumigatus 58128	LGDEVAANFT	ALFAPdIRAR	aEkhlPGVtL TDEDVVS LMD MCSFDtVArT
A. fumigatus 26906	LGDEVAANFT	ALFAPdIRAR	aKkhLPGVtL TDEDVVS LMD MCSFDtVArT
A. fumigatus 32239	LGDEVEANFT	ALFAPAIRAR	IEkhLPGVQL TDDDVVS LMD MCSFDtVArT
E. nidulans	rADEIEANFT	AIMGPPIRkR	LEndLPGIKL TNENVIyLMD MCSFDtMArT
T. thermophilus	gGHDaQEKFA	kqFAPAIleK	IKDhLPGVDL AvsDVpyLMD LCPFETLArN
T. lanuginosa	.DptqpAEFl	qVFGPRVlkK	ItkhMPGVNL TLEDVplFMD LCPFDtVGsd
M. thermophila	IGDDaQDtYl	StFAGPitAR	VNAnLPGaNL TDADtVaLMD LCPFETVAsS
Basidio	dSDpqxnXWl	AVFAPPitAR	LNAaaPGaNL TDxDaxNLxx LCPFETVS..

Consensus	LGDDVEANFT	AVFAPPIRAR	LEA-LPGVNL	TDEDVVNLMD	MCPFDtVA-T
Fcp10	LGDDVEANFT	AVFAPPIRAR	LEAHLPGVNL	TDEDVVNLMD	MCPFDtVART

	251		300
A. terreus 9a1	dD..Aht...LSPF	CDLFTa...te WtQYNYLlSL dKYYGYGGGN
A. terreus cbs	dD..Aht...LSPF	CDLFTa...ae WtQYNYLlSL dKYYGYGGGN
A. niger var. awamori	Tv..DTK...LSPF	CDLFTH..de WiHYDYlQSL kKYYGHGAGN
A. niger NRRL3135	Tv..DTK...LSPF	CDLFTH..de WiNYDYlQSL kKYYGHGAGN
A. fumigatus 13073	SD..ASQ...LSPF	QQLFTH..ne WkKYNyLQSL gKYYGYGAGN
A. fumigatus 32722	SD..ASQ...LSPF	QQLFTH..ne WkKYNyLQSL gKYYGYGAGN
A. fumigatus 58128	SD..ASQ...LSPF	QQLFTH..ne WkKYNyLQSL gKYYGYGAGN
A. fumigatus 26906	SD..ASQ...LSPF	QQLFTH..ne WkKYNyLQSL gKYYGYGAGN
A. fumigatus 32239	AD..ASE...LSPF	CAIFTH..ne WkKYDYlQSL gKYYGYGAGN
E. nidulans	AH..GTE...LSPF	CAIFTE..ke WlQYDYlQSL sKYYGYGAGS
T. thermophilus	ht..DT...LSPF	CALStQ...e WqaYDYyQSL gKYYGnGGGN
T. lanuginosa	PvlfPrQ...LSPF	CHLFTa...d WmaYDYyTL dKYYSHGGGS
M. thermophila	SsdPaTadag	ggngRpLSPF	CrLFSE...e WraYDYlQSV gKWYGYGPgN
BasidioxexxSxF	CDLFexxpeE FxaFxYxgdL dKFYGTgYgQ

Consensus	SD--ATQ---	-----LSPF	CDLFTH---E	W-QYDYlQSL	-KYYGYGAGN
Fcp10	SD..ATQ...LSPF	CDLFTH..DE	WlQYDYlQSL	gKYYGYGAGN

	301		350
<i>A. terreus</i> 9a1	PLGPPvQGVGW	aNELMARLTR	A. PVHDHTCv NNTLDASPAT FPLNATLYAD
<i>A. terreus</i> cbs	PLGPPvQGVGW	aNELIARLTR	S. PVHDHTCv NNTLDANPAT FPLNATLYAD
<i>A. niger</i> var. <i>awamori</i>	PLGPTQGVGY	aNELIARLTH	S. PVHDDTSS NHTLDSNPAT FPLNSTLYAD
<i>A. niger</i> NRRL3135	PLGPTQGVGY	aNELIARLTH	S. PVHDDTSS NHTLDSNPAT FPLNSTLYAD
<i>A. fumigatus</i> 13073	PLGPAQGGIGF	tNELIARLTR	S. PVQDHTST NsTLvSNPAT FPLNATMYvD
<i>A. fumigatus</i> 32722	PLGPAQGGIGF	tNELIARLTR	S. PVQDHTST NsTLvSNPAT FPLNATMYvD
<i>A. fumigatus</i> 58128	PLGPAQGGIGF	tNELIARLTR	S. PVQDHTST NsTLvSNPAT FPLNATMYvD
<i>A. fumigatus</i> 26906	PLGPAQGGIGF	tNELIARLTR	S. PVQDHTST NsTLvSNPAT FPLNATMYvD
<i>A. fumigatus</i> 32239	PLGPAQGGIGF	tNELIARLTH	S. PVQDHTST NsTLvSNPAT FPLNATMYvD
<i>E. nidulans</i>	PLGPAQGGIGF	tNELIARLTQ	S. PVQDNTST NHTLDSNPAT FPLDRLKYAD
<i>T. thermophilus</i>	PLGPAQGVGF	vNELIARMTg	S. PVQDYTTv NHTLDSNPAT FPLNATLYAD
<i>T. lanuginosa</i>	AFGPPSRGVGF	vNELIARMTg	NLPVKDHTTv NHTLDdNPET FPLDAvLYAD
<i>M. thermophila</i>	PLGPTQGVGF	vNELLARLA	GvPVRDgTST NRTLDGDPRT FPLGrPLYAD
Basidio	PLGPPvQGVGY	iNELIARLTx	qa. VRDNTqT NRTLDSSpXT FPLNtTFYAD

Consensus PLGPAQGVGF -NELIARLTH S-PVQDHTST NHTLDSNPAT FPLNATLYAD
 Fcp10 PLGPAQGVGF vNELIARLTH S. PVQDHTST NHTLDSNPAT FPLNATLYAD

	351		400
<i>A. terreus</i> 9a1	FSHDSnLVSI	FWALGLYNGT	aPLSqtSVE. .SvsQTDGYA AAWTVPPFAAR
<i>A. terreus</i> cbs	FSHDSnLVSI	FWALGLYNGT	kPLSqtTVE. .ditrTDGYA AAWTVPPFAAR
<i>A. niger</i> var. <i>awamori</i>	FSHDNGIISI	LFALGLYNGT	kPLSTTTVE. .NitQTDGFS SAWTVPPFASR
<i>A. niger</i> NRRL3135	FSHDNGIISI	LFALGLYNGT	kPLSTTTVE. .NitQTDGFS SAWTVPPFASR
<i>A. fumigatus</i> 13073	FSHDNSMVSI	FFALGLYNGT	ePLSrTSVE. .SaKEIDGYS ASWvVPPGAR
<i>A. fumigatus</i> 32722	FSHDNSMVSI	FFALGLYNGT	gPLSrTSVE. .SaKEIDGYS ASWvVPPGAR
<i>A. fumigatus</i> 58128	FSHDNSMVSI	FFALGLYNGT	ePLSrTSVE. .SaKEIDGYS ASWvVPPGAR
<i>A. fumigatus</i> 26906	FSHDNSMVSI	FFALGLYNGT	ePLSrTSVE. .SaKEIDGYS ASWvVPPGAR
<i>A. fumigatus</i> 32239	FSHDNGMIPI	FFAMGLYNGT	ePLSqtSeE. .StKESNGYS ASWAVPPGAR
<i>E. nidulans</i>	FSHDNSMISI	FFAMGLYNGT	qPLSmdSVE. .SiQEmDGYA ASWTVPPGAR
<i>T. thermophilus</i>	FSHDNTMTGI	FaALGLYNGT	akLSTTeIK. .SiEETDGYS AAWTVPPFASR
<i>T. lanuginosa</i>	FSHDNTMTGI	FsAMGLYNGT	kPLSTSkIQP ptgAAADGYA ASWTVPPFAAR
<i>M. thermophila</i>	FSHDNDMMGV	LgALGaYDGV	pPLdkTA. .R rdpEEIGGYA ASWAVPPFAAR
Basidio	FSHDNqMVAI	FsAMGLFNqS	aPLdPSxpDP nrt.Wv TSklVPFSAR

Consensus FSHDNTMVSI FFALGLYNGT -PLSTTSVEP -S-EETDGYS ASWTVPPFAAR
 Fcp10 FSHDNTMVSI FFALGLYNGT KPLSTTSVE. .SiEETDGYS ASWTVPPFAAR

	401		450
<i>A. terreus</i> 9a1	AYVEMMQC..	ra.....EKEPL VRVLVNDVRM PLHGCPtDKL
<i>A. terreus</i> cbs	AYIEMMQC..	ra.....EKQPL VRVLVNDVRM PLHGCAVDNL
<i>A. niger</i> var. <i>awamori</i>	lyVEMMQC..	Qa.....EQEPL VRVLVNDRVV PLHGCPIDaL
<i>A. niger</i> NRRL3135	lyVEMMQC..	Qa.....EQEPL VRVLVNDRVV PLHGCPVDaL
<i>A. fumigatus</i> 13073	AYfEtMQC..	Ks.....EKEPL VRaLINDRVV PLHGCDVDKL
<i>A. fumigatus</i> 32722	AYfEtMQC..	Ks.....EKEPL VRaLINDRVV PLHGCDVDKL
<i>A. fumigatus</i> 58128	AYfEtMQC..	Ks.....EKESL VRaLINDRVV PLHGCDVDKL
<i>A. fumigatus</i> 26906	AYfEtMQC..	Ks.....EKEPL VRaLINDRVV PLHGCDVDKL
<i>A. fumigatus</i> 32239	AYfEtMQC..	Ks.....EKEPL VRaLINDRVV PLHGCAVDKL
<i>E. nidulans</i>	AYfELMQC..	E.....KKEPL VRVLVNDRVV PLHGCAVDKf
<i>T. thermophilus</i>	AYIEMMQC..	Dd.....sDEPV VRVLVNDRVV PLHGCEVdSL
<i>T. lanuginosa</i>	AYVELLRC..	Etetsseeee	EG...EDEPF VRVLVNDRVV PLHGCrVDRW
<i>M. thermophila</i>	iyVEkMRC..	sggggggggg	EGrqeKDEeM VRVLVNDVRM TLkGCGaDer
Basidio	mvVerLxCxx	xgtxxxxxxx	xxxxxxxxxxx VRVLVNDaVq PLEfCGgDxd

Consensus AYVEMMQC-- E----- EG---EKEPL VRVLVNDRVV PLHGCGVDKL
 Fcp10 AYVEMMQC.. EA..... EKEPL VRVLVNDRVV PLHGCGVDKL

	451	482
<i>A. terreus</i> 9a1	GRCKrDAFVA GLSFAQAG..	GNWADCF--- --
<i>A. terreus</i> cbs	GRCKrDDFVE GLSFARAG..	GNWAECF--- --
<i>A. niger</i> var. <i>awamori</i>	GRCtrDsFVr GLSFARSG..	GDWAECsA-- --
<i>A. niger</i> NRRL3135	GRCtrDsFVr GLSFARSG..	GDWAECFA-- --
<i>A. fumigatus</i> 13073	GRCKlNDFVK GLSWARSG..	GNWGECS-- --
<i>A. fumigatus</i> 32722	GRCKlNDFVK GLSWARSG..	GNWGECS-- --
<i>A. fumigatus</i> 58128	GRCKlNDFVK GLSWARSG..	GNWGECS-- --
<i>A. fumigatus</i> 26906	GRCKlNDFVK GLSWARSG..	GNWGECS-- --
<i>A. fumigatus</i> 32239	GRCKlKDFVK GLSWARSG..	GNSEQSFS-- --
<i>E. nidulans</i>	GRCtlDDWVE GLNFARSG..	GNWktCFT1~ --
<i>T. thermophilus</i>	GRCKrDDFVr GLSFARqG..	GNWEGCYAas e-
<i>T. lanuginosa</i>	GRCRrDEWIK GLTFARqG..	GHWDrCF--- --
<i>M. thermophila</i>	GmCtlErFIE SMAFARGN..	GKWDlCFA-- --
Basidio	GxCtlDAFVE SqxYAReDgq	GDFEKCFAtp xx
Consensus	GRCK-DDFVE GLSFARSG--	GNWEECF-- --
Fcp10	GRCKRDDFVE GLSFARSG..	GNWEECF.. ..

Figure 5

CP-1
 Eco RI M G V F V V L L S I A T L F G S T 17
 TATATGAATTCA~~TGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTTCGGTTCCA~~
 1 -----+-----+-----+-----+-----+ 60
 ATATACTTAAGTACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAAACAAGCCAAGGT

 S G T A L G P R G N S H S C D T V D G G 37
 CATCCGGTACCGCCTTGGGTCCTCGTGGAATTCTCACTCTTGACACTGTTGACGGTG
 61 -----+-----+-----+-----+-----+ 120
 GTAGGCCATGGCGGAACCCAGGAGCACCATTAAAGAGTGAGAACACTGTGACAACCTGCCAC
 CP-2
 CP-3.10
 Y Q C F P E I S H L W G Q Y S P E F S L 57
 GTTACCAATGTTTCCCAGAAATTTCTCACTTGTGGGGTCAATACTCTCCATTCTTCTCTT
 121 -----+-----+-----+-----+-----+ 180
 CAATGGTTACAAAGGGTCTTTAAAGAGTGAACACCCCAGTTATGAGAGGTAAGAAGAGAA

 A D E S A I S P D V P K G C R V T F V Q 77
 TGGCTGACGAATCTGCTATTTCTCCAGACGTTCCAAAGGGTGTAGAGTTACTTTCGTTC
 181 -----+-----+-----+-----+-----+ 240
 ACCGACTGCTTAGACGATAAAGAGGTCTGCAAGGTTTCCCGACATCTCAATGAAAGCAAG
 CP-4.10
 CP-5.10
 V L S R H G A R Y P T S S K S K K Y S A 97
 AAGTTTTGTCTAGACACGGTGCTAGATACCCAACTTCTTCTAAGTCTAAGAAGTACTCTG
 241 -----+-----+-----+-----+-----+ 300
 TTCAAAACAGATCTGTGCCACGATCTATGGGTTGAAGAAGATTGAGATTCTTCATGAGAC

 L I E A I Q K N A T A F K G K Y A F L K 117
 CTTTGATTGAAGCTATTCAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGA
 301 -----+-----+-----+-----+-----+ 360
 GAAACTAACTTCGATAAGTTTTCTTGGCGATGACGAAAGTTCCCATTCATGCGAAAGAACT
 CP-6
 CP-7.10
 T Y N Y T L G A D D L T P F G E Q Q M V 137
 AGACTTACAACCTACACTTTGGGTGCTGACGACTTGACTCCATTCCGGTGAACAACAAATGG
 361 -----+-----+-----+-----+-----+ 420
 TCTGAATGTTGATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTGTTGTTTACC

 N S G I K F Y R R Y K A L A R K I V P F 157
 TTAACCTCTGGTATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCAT
 421 -----+-----+-----+-----+-----+ 480
 AATTGAGACCATAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTA
 CP-8.10
 CP-9.10
 Y R A S G S D R V I A S A E K F I E G F 177
 TCGTTAGAGCTTCTGGTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTTCATTGAAGGTT
 481 -----+-----+-----+-----+-----+ 540
 AGCAATCTCGAAGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAA

 Q S A K L A D P G A N P H Q A S P V I N 197
 TCCAATCTGCTAAGTTGGCTGACCCAGGTGCTAACCCACACCAAGCTTCTCCAGTTATTA
 541 -----+-----+-----+-----+-----+ 600
 AGGTTAGACGATTCAACCGACTGGGTCCACGATTGGGTGTGGTTCGAAGAGGTCAATAAT

CP-10.10CP-11.10

V I I P E G A G Y N N T L D H G L C T A 217
 ACGTTATTATTCCAGAAGGTGCTGGTTACAACAACACTTTGGACCACGGTTTGTGTACTG
 601 -----+-----+-----+-----+-----+ 660
 TGCAATAATAAGGTCTTCCACGACCAATGTTGTTGTGAAACCTGGTGCCAAACACATGAC

F E E S E L G D D V E A N F T A V F A P 237
 CTTTCGAAGAATCTGAATTGGGTGACGACGTTGAAGCTAACTTCACTGCTGTTTTTCGCTC
 661 -----+-----+-----+-----+-----+ 720
 GAAAGCTTCTTAGACTTAACCCACTGCTGCAACTTCGATTGAAGTGACGACAAAAGCGAG

CP-12.10

P I R A R L E A H L P G V N L T D E D V 257
 CACCTATTAGAGCTAGATTGGAAGCTCACTTGCCAGGTGTTAACTTGACTGACGAAGACG
 721 -----+-----+-----+-----+-----+ 780
 GTGGATAATCTCGATCTAACCTTCGAGTGAACGGTCCACAATTGAACTGACTGCTTCTGC

CP-13.10

V N L M D M C P F D T V A R T S D A T Q 277
 TTGTTAACTTGATGGACATGTGTCCATTTCGACACTGTTGCTAGAACTTCTGACGCTACTC
 781 -----+-----+-----+-----+-----+ 840
 AACAAATGAACTACCTGTACACAGGTAAGCTGTGACAACGATCTTGAAGACTGCGATGAG

L S P F C D L F T H D E W I Q Y D Y L Q 297
 AATTGTCTCCATTCTGTGACTTGTTCCTCAACGACGAATGGATTCAATACGACTACTTGC
 841 -----+-----+-----+-----+-----+ 900
 TTAACAGAGGTAAGACACTGAACAAGTGAGTGCTGCTTACCTAAGTTATGCTGATGAACG

CP-14.10CP-15.10

S L G K Y Y G Y G A G N P L G P A Q G V 317
 AATCTTTGGGTAAAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTG
 901 -----+-----+-----+-----+-----+ 960
 TTAGAAAACCCATTTCATGATGCCAATGCCACGACCATTTGGGTAAACCCAGGTGAGTTCCAC

G F V N E L I A R L T H S P V Q D H T S 337
 TTGGTTTCGTTAACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTT
 961 -----+-----+-----+-----+-----+ 1020
 AACCAAAGCAATTGCTTAACTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAA

CP-16.10CP-17.10

T N H T L D S N P A T F P L N A T L Y A 357
 CTACTAACCACACTTTGGACTCTAACCCAGCTACTTTCCATTGAACGCTACTTTGTACG
 1021 -----+-----+-----+-----+-----+ 1080
 GATGATTGGTGTGAAACCTGAGATTGGGTGATGAAAGGGTAACTTGCGATGAAACATGC

D F S H D N I M Y S I F F A L G L Y N G 377
 CTGACTTCTCTCAGACAACACTATGGTTTCTATTTTCTTCGCTTTGGGTTTGTACAACG
 1081 -----+-----+-----+-----+-----+ 1140
 GACTGAAGAGAGTGCTGTTGTGATACCAAAGATAAAGAAGCGAAACCCAAACATGTTGC

CP-18.10CP-19.10

T K P L S T T S V E S I E E T D G Y A A 397
 GTACTAAGCCATTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTTACGCTG
 1141 -----+-----+-----+-----+-----+ 1200
 CATGATTCCGGTAACAGATGATGAAGACAACCTTAGATAACTTCTTTGACTGCCAATGCGAC

S W T V P F A A R A Y V E M M Q C E A E 417
CTTCTTGGAAGTGTTCATTGCTGCTAGAGCTTACGTTGAAATGATGCAATGTGAAGCTG
1201 -----+-----+-----+-----+-----+-----+ 1260
GAAGAACCTGACAAGGTAAGCCACGATCTCGAATGCAACTTTACTACGTTACACTTCGAC
CP-20.10
CP-21.10
K E P L V R V L V N D R V V P L H G C G 437
AAAAGGAACCATTTGGTTAGAGTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTG
1261 -----+-----+-----+-----+-----+-----+ 1320
TTTTCCTTGGTAACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACAC
V D K L G R C K R D D F V E G L S F A R 457
GTGTTGACAAGTTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTTCGCTA
1321 -----+-----+-----+-----+-----+-----+ 1380
CACAACTGTTCAACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGAT
CP-22.10
S G G N W E E C F A * Eco RI 467
GATCTGGTGGTAAGTGGGAAGAATGTTTCGCTTAAGAATTCATATA
1381 -----+-----+-----+-----+-----+-----+ 1426
CTAGACCACCATTGACCCTTCTTACAAAGCGAATTCTTAAGTATAT

Figure 6

	1		50
<i>P. involutus</i> (phyA1)	-----	-FPipeseqR nWSPYSPYFP LAEyKA....	pPaGCQInqV
<i>P. involutus</i> (phyA2)	-----	-FsipeseqR nWSPYSPYFP LAEyKA....	pPaGCeInqV
<i>T. pubescens</i>	-----	-LDvtRDVqQ sWSmYSPYFP aAtyvA....	pPaSCQInqV
<i>A. pediades</i>	-----	-pffpPQIQD sWAaYTPYYP VqAyTP....	pPKDCKITqV
<i>P. lycii</i>	-----	-LPipAQnTs nWGPYdPFFP VEpyAA....	pPEGCTVTqV
<i>A. terreus</i> 9a1	KhSDCNSVDh	GYQcfPELSH kWGLYAPYFS LqDESFPFID	VPEDCHITFV
<i>A. terreus</i> cbs	NhSDCtSVDr	GYQcfPELSH kWGLYAPYFS LqDESFPFID	VPDDCHITFV
<i>A. niger</i> var. <i>awamori</i>	NqSTCDTVDq	GYQcfSEtSH LWGQYAPFFS LANESAISPD	VPaGCRVTFa
<i>A. niger</i> T213	NqSsCDTVDq	GYQcfSEtSH LWGQYAPFFS LANESvISPD	VPaGCRVTFa
<i>A. niger</i> NRRL3135	NqSsCDTVDq	GYQcfSEtSH LWGQYAPFFS LANESvISPE	VPaGCRVTFa
<i>A. fumigatus</i> ATCC13073	GSKsCDTVD1	GYQcSPAtSH LWGQYSPFFS LEDElSVSSK	LPKDCRITLV
<i>A. fumigatus</i> ATCC32722	GSKsCDTVD1	GYQcSPAtSH LWGQYSPFFS LEDElSVSSK	LPKDCRITLV
<i>A. fumigatus</i> ATCC58128	GSKsCDTVD1	GYQcSPAtSH LWGQYSPFFS LEDElSVSSK	LPKDCRITLV
<i>A. fumigatus</i> ATCC26906	GSKsCDTVD1	GYQcSPAtSH LWGQYSPFFS LEDElSVSSK	LPKDCRITLV
<i>A. fumigatus</i> ATCC32239	GSKACDTVEl	GYQcSPGtSH LWGQYSPFFS LEDElSVSSD	LPKDCRVTFV
<i>E. nidulans</i>	QNHSCNTaDg	GYQcFPNVSH VWGQYSPYFS IEQESAISed	VPhGCeVTFV
<i>T. thermophilus</i>	DSHSCNTVEg	GYQCrPEISH sWGQYSPFFS LADQSEISPD	VPQNCKITFV
<i>T. lanuginosa</i>	-----	nvDIAR hWGQYSPFFS LAEvSEISPA	VPKGCrvEFV
<i>M. thermophila</i>	ESRPCDTpDl	GFQCgTAISH FWGQYSPYFS VPSElDaS..	IPDDCeVTFa
Consensus Seq. 11	NSHSCDTVD-	GYQC-PEISH LWGQYSPFFS LADESAISPD	VPKGCrvTFV
	51		100
<i>P. involutus</i> (phyA1)	NIiQRHGARF	PTSGaTtRik AgLtKLQgvq nftDAKFNF	KSFkydLGns
<i>P. involutus</i> (phyA2)	NIiQRHGARF	PTSGaAtRik AgLsKLQsvq nftDPKFDFI	KSFtydLGts
<i>T. pubescens</i>	HIiQRHGARF	PTSGaAKRiq TaVAKLKaaS nytDPILAFV	tnYtYSLGqD
<i>A. pediades</i>	NIiQRHGARF	PTSGaGtRiq AaVKKLQsak TytDPRLDfL	tnYtYTLGhd
<i>P. lycii</i>	NLIQRHGARW	PTSGarsRqv AaVAKIQmar PftDPKYEFL	NdFvYkFGvA
<i>A. terreus</i> 9a1	QVLARHGARS	PTHsKTKaYA AtIAaIQKSA TaFpGKYAFL	QSYNYSLDSE
<i>A. terreus</i> cbs	QVLARHGARS	PTdSKTKaYA AtIAaIQKNA TaLpGKYAFL	KSYNYSMGSE
<i>A. niger</i> var. <i>awamori</i>	QVLSRHGARY	PTeSKGKKYS ALIEeIQQNv TtFDGKYAFL	KTYNYSLGAD
<i>A. niger</i> T213	QVLSRHGARY	PTeSKGKKYS ALIEeIQQNv TtFDGKYAFL	KTYNYSLGAD
<i>A. niger</i> NRRL3135	QVLSRHGARY	PTdSKGKKYS ALIEeIQQNA TtFDGKYAFL	KTYNYSLGAD
<i>A. fumigatus</i> ATCC13073	QVLSRHGARY	PTSSKSKKYk kLVtaIQaNA TdFKGKFAFL	KTYNYTLGAD
<i>A. fumigatus</i> ATCC32722	QVLSRHGARY	PTSSKSKKYk kLVtaIQaNA TdFKGKFAFL	KTYNYTLGAD
<i>A. fumigatus</i> ATCC58128	QVLSRHGARY	PTSSKSKKYk kLVtaIQaNA TdFKGKFAFL	KTYNYTLGAD
<i>A. fumigatus</i> ATCC26906	QVLSRHGARY	PTSSKSKKYk kLVtaIQaNA TdFKGKFAFL	KTYNYTLGAD
<i>A. fumigatus</i> ATCC32239	QVLSRHGARY	PTASKSKKYk kLVtaIQKNA TeFKGKFAFL	ETNYNYTLGAD
<i>E. nidulans</i>	QVLSRHGARY	PTeSKSKaYS GLIEaIQKNA TsFwGQYAF	ESYNYTLGAD
<i>T. thermophilus</i>	QLLSRHGARY	PTSSKTElYS qLIeRIQKtA TaYKGyYAFL	KdYrYqLGAN
<i>T. lanuginosa</i>	QVLSRHGARY	PTAhKSEvYA ELLQRIQDtA TeFKGDFAFL	RdYaYhLGAD
<i>M. thermophila</i>	QVLSRHGARA	PTlkRAasYv DLIDRIHhGA isYgPgYEFL	RTYDYTLGAD
Consensus Seq. 11	QVLSRHGARY	PTSSKSKKYs ALIERIQKNA T-FKGKYAFL	KTYNYTLGAD

	101	150
<i>P. involutus</i> (phyA1)	DLvPFGAaQs fDAGqEaFaR YskLvSKNnL PFIRAdGSDR VVDSAtNwTA	
<i>P. involutus</i> (phyA2)	DLvPFGAaQs fDAGLevFaR YskLvSeDnL PFIRsdGSDR VVDTAtNwTA	
<i>T. pubescens</i>	sLveLGAtQs sEAGqEaFtR YsSLvSaDeL PFVRASGSDR VVATANNwTA	
<i>A. pediades</i>	DLvPFGAlQs sQAGeEtFQR YsfLvSKEnL PFVRASSSNR VVDSAtNwTE	
<i>P. lycii</i>	DLlPFGANQs hQTGTDMYtR YsTLfEgGdV PFVRAAGdQR VVDSStNwTA	
<i>A. terreus</i> 9a1	ELTPFGGrNQL rDlGaQFYeR YNAL.TRHIn PFVRATDAeR VhESAeKFVE	
<i>A. terreus</i> cbs	NLTPFGGrNQL qDlGaQFYRR YDTL.TRHIn PFVRAADSsR VhESAeKFVE	
<i>A. niger</i> var. <i>awamori</i>	DLTPFGEQEL VNSGIKFYQR YESL.TRNII PFIRSSGSsR VIASGEKFIE	
<i>A. niger</i> T213	DLTPFGEQEL VNSGIKFYQR YESL.TRNII PFIRSSGSsR VIASGEKFIE	
<i>A. niger</i> NRRL3135	DLTPFGEQEL VNSGIKFYQR YESL.TRNIV PFIRSSGSsR VIASGKKFIE	
<i>A. fumigatus</i> ATCC13073	DLTPFGEQQL VNSGIKFYQR YKAL.ARSV V PFIRASGSDR VIASGEKFIE	
<i>A. fumigatus</i> ATCC32722	DLTPFGEQQL VNSGIKFYQR YKAL.ARSV V PFIRASGSDR VIASGEKFIE	
<i>A. fumigatus</i> ATCC58128	DLTPFGEQQL VNSGIKFYQR YKAL.ARSV V PFIRASGSDR VIASGEKFIE	
<i>A. fumigatus</i> ATCC26906	DLTAFGEQQL VNSGIKFYQR YKAL.ARSV V PFIRASGSDR VIASGEKFIE	
<i>A. fumigatus</i> ATCC32239	DLTPFGEQQL VNSGIKFYQK YKAL.AgSVV PFIRSSGSsR VIASGEKFIE	
<i>E. nidulans</i>	DLTiFGENQM VDSGaKfYRR YKnL.ARKnt PFIRASGSDR VVASAEKFIn	
<i>T. thermophilus</i>	DLTPFGENQM IQlGIKFYnH YKSL.ARNv PFVRCGSDR VIASGrIFIE	
<i>T. lanuginosa</i>	NLTRFGEEQM MESGrQFYHR YREq.AREIV PFVRAAGSAR VIASAEfFnr	
<i>M. thermophila</i>	ELTRtGQQQM VNSGIKFYRR YRAL.ARKsI PFVRTAGqDR VVhSAENFtQ	

Consensus Seq. 11 DLTPFGENQM VNSGIKFYRR YKAL-ARNIV PFVRASGSDR VIASAEKFIE

	151	200
<i>P. involutus</i> (phyA1)	GFaSA.....shNtvqPk LNLILPQ..T gNDTLEDNMC PAaGD.....	
<i>P. involutus</i> (phyA2)	GFaSA.....srNaiqPk LDLILPQ..T gNDTLEDNMC PAaGE.....	
<i>T. pubescens</i>	GFaLA.....ssNsitPV LSVIIE..A gNDTLDDNMC PAaGD.....	
<i>A. pediades</i>	GFsAA.....shHvlnPI LfVILSE..S LNDTLDDAMC PnaGs.....	
<i>P. lycii</i>	GFgdA.....sgEtvlpT LQVVLQE..E gNcTLcNNMC PnevD.....	
<i>A. terreus</i> 9a1	GFQTARqDDh hAnpHQPSPr VDVAIPEGSA YNNTLEHSLC TAFES...ST	
<i>A. terreus</i> cbs	GFQNaRqGDP hAnpHQPSPr VDVVIPEGTA YNNTLEHSIC TAFEa...ST	
<i>A. niger</i> var. <i>awamori</i>	GFQSTKLkDP rAqpgQSSPk IDVVISeASS sNNTLDpGtC TvFED...Se	
<i>A. niger</i> T213	GFQSTKLkDP rAqpgQSSPk IDVVISeASS sNNTLDpGtC TvFED...Se	
<i>A. niger</i> NRRL3135	GFQSTKLkDP rAqpgQSSPk IDVVISeASS sNNTLDpGtC TvFED...Se	
<i>A. fumigatus</i> ATCC13073	GFQqAKLADP gAt.NRAAPa ISVIIPeSeT FNNTLDHGVC TkFEa...Sq	
<i>A. fumigatus</i> ATCC32722	GFQqAKLADP gAt.NRAAPa ISVIIPeSeT FNNTLDHGVC TkFEa...Sq	
<i>A. fumigatus</i> ATCC58128	GFQqAKLADP gAt.NRAAPa ISVIIPeSeT FNNTLDHGVC TkFEa...Sq	
<i>A. fumigatus</i> ATCC26906	GFQqAKLADP gAt.NRAAPa ISVIIPeSeT FNNTLDHGVC TkFEa...Sq	
<i>A. fumigatus</i> ATCC32239	GFQqANVADP gAt.NRAAPV ISVIIPeSeT YNNTLDHSVC TnFEa...Se	
<i>E. nidulans</i>	GFRkaQLhDh g.s.gQATPV VNVIIPEIdG FNNTLDHStC vSFEN...de	
<i>T. thermophilus</i>	GFQSAKVLDP hSdKHdAPpt INVIIeEGPS YNNTLDtGSc PvFED...SS	
<i>T. lanuginosa</i>	GFQdAKdrDP rSnkDQaEPV INVIISeETG sNNTLDgltC PAaEE...AP	
<i>M. thermophila</i>	GFHSaLLADR gStvRPTlPy dmVVIPEtAG aNNTLHNDLC TAFEegpyST	

Consensus Seq. 11 GFQSAKLADP -A--HQASPV INVIIPEGSG YNNTLDHGVC TAFED---ST

	201		250
<i>P. involutus</i> (phyA1)	.SDpqvnaWl	AVafPSItAR	LNAaaPSVNL TdtDafNLVs LCAF1TVSK.
<i>P. involutus</i> (phyA2)	.SDpqvDaWl	AsafPSVtAQ	LNAaaPGaNL TDADafNLVs LCPFMtVSK.
<i>T. pubescens</i>	.SDpqvnQWl	AqFAPPMtAR	LNagaPGaNL TdtDtyNLLt LCPFETVat.
<i>A. pediades</i>	.SDpqtGiWT	SIYGTPIanR	LNqqaPGaNI TAADVsnLIp LCAFETIvK.
<i>P. lycii</i>	.GDESt.tWl	GVFAPnItAR	LNAaaPSaNL SdsDaLtLMD MCPFDtLSs.
<i>A. terreus</i> 9a1	VGDDAvANFT	AVFAPAIaqR	LEAdLPGVQL StDDVVNLMA MCPFETVSlT
<i>A. terreus</i> cbs	VGDAADNFT	AVFAPAIakR	LEAdLPGVQL SADDVVNLMA MCPFETVSlT
<i>A. niger</i> var. <i>awamori</i>	LADtveANFT	AtFAPSIRqR	LEndLSGVtL TdtEVtyLMD MCSFDtIstS
<i>A. niger</i> T213	LADtveANFT	AtFAPSIRqR	LEndLSGVtL TdtEVtyLMD MCSFDtIstS
<i>A. niger</i> NRRL3135	LADtveANFT	AtFvPSIRqR	LEndLSGVtL TdtEVtyLMD MCSFDtIstS
<i>A. fumigatus</i> ATCC13073	LGDEvAANFT	ALFAPdIRAR	aEkhlPGVtL TDEDVVS LMD MCSFDtVART
<i>A. fumigatus</i> ATCC32722	LGDEvAANFT	ALFAPdIRAR	aEkhlPGVtL TDEDVVS LMD MCSFDtVART
<i>A. fumigatus</i> ATCC58128	LGDEvAANFT	ALFAPdIRAR	aEkhlPGVtL TDEDVVS LMD MCSFDtVART
<i>A. fumigatus</i> ATCC26906	LGDEvAANFT	ALFAPdIRAR	aKkhLPGVtL TDEDVVS LMD MCSFDtVART
<i>A. fumigatus</i> ATCC32239	LGDEvAANFT	ALFAPdIRAR	IEkhLPGVQL TDDDVVS LMD MCSFDtVART
<i>E. nidulans</i>	rADEiEANFT	AIMGPPIRkR	LEndLPGIKL TNENViYlMD MCSFDtMART
<i>T. thermophilus</i>	gGHDAQEKFA	kqFAPAIeK	IKDhLPGVDL AvsDVpyLMD LCPFETLARN
<i>T. lanuginosa</i>	.DptqpAEFl	qVFGPRVlKk	ItkhMPGVNL TLEDVplFMD LCPFDtVGsd
<i>M. thermophila</i>	IGDDAQDtYl	StFAGPItAR	VNanLPGaNL TDADtVaLMD LCPFETVAss

Consensus Seq. 11 LGDDAEANFT AVFAPPiRAR LEA-LPGVNL TDEDVVNLMD MCPFDtVART

	251		300
<i>P. involutus</i> (phyA1)ekkSdF	CtLFegIPGs FeaFAYggdL dKfYgtGyGQ
<i>P. involutus</i> (phyA2)eqkSdF	CtLFegIPGs FeaFAYagdL dKfYgtGyGQ
<i>T. pubescens</i>errSeF	CDIYeelqAE .daFAYnadL dKfYgtGyGQ
<i>A. pediades</i>etpSPF	CNLF..TPEE FaQFEYFgdL dKfYgtGyGQ
<i>P. lycii</i>gnaSPF	CDLF..TAEe YvsYEYYydl dKYYGtGPGN
<i>A. terreus</i> 9a1	dD..Aht...LSPF	CDLF..TAtE WtQYNYLlSL dKYYGYGGGN
<i>A. terreus</i> cbs	dD..Aht...LSPF	CDLF..TAAE WtQYNYLlSL dKYYGYGGGN
<i>A. niger</i> var. <i>awamori</i>	Tv..DTK...LSPF	CDLF..ThDE WiHYDYlQSL kKYYGHGAGN
<i>A. niger</i> T213	Tv..DTK...LSPF	CDLF..ThDE WiHYDYlRSL kKYYGHGAGN
<i>A. niger</i> NRRL3135	Tv..DTK...LSPF	CDLF..ThDE WiNYDYlQSL kKYYGHGAGN
<i>A. fumigatus</i> ATCC13073	SD..ASQ...LSPF	CQLF..ThNE WkKYNyLQSL gKYYGYGAGN
<i>A. fumigatus</i> ATCC32722	SD..ASQ...LSPF	CQLF..ThNE WkKYNyLQSL gKYYGYGAGN
<i>A. fumigatus</i> ATCC58128	SD..ASQ...LSPF	CQLF..ThNE WkKYNyLQSL gKYYGYGAGN
<i>A. fumigatus</i> ATCC26906	SD..ASQ...LSPF	CQLF..ThNE WkKYNyLQSL gKYYGYGAGN
<i>A. fumigatus</i> ATCC32239	AD..ASE...LSPF	CAIF..ThNE WkKYDYlQSL gKYYGYGAGN
<i>E. nidulans</i>	AH..GTE...LSPF	CAIF..TEKE WlQYDYlQSL sKYYGYGAGS
<i>T. thermophilus</i>	ht..DT....LSPF	CALs..TqEE WqaYDYyQSL gKYYGnGGGN
<i>T. lanuginosa</i>	PvlfPrQ...LSPF	CHLF..TADD WmaYDYyTL dKYYSHGGGS
<i>M. thermophila</i>	SsdpAtadag	ggngnrLSPF	CrLF..SEsE WraYDYlQSV gKYYGYGPGN

Consensus Seq. 11 SD--ATQ--- -----LSPF CDLF--TADE W-QYDYlQSL -KYYGYGAGN

	301		350
<i>P. involutus</i> (phyA1)	eLGPvQGVGY vNELIARLTN	S.AVRDNTqT	NRTLDAASPvT FPLNkTFYAD
<i>P. involutus</i> (phyA2)	ALGPvQGVGY iNELIARLTN	S.AVNDNTqT	NRTLDAApDT FPLNkTMYAD
<i>T. pubescens</i>	PLGPvQGVGY iNELIARLTa	q.nVsDHTqT	NeTLdSSPET FPLNrTLYAD
<i>A. pediades</i>	PLGPvQGVGY iNELIARLTa	m.PVRDNTqT	NRTLdSSPlT FPLDrSIYAD
<i>P. lycii</i>	ALGPvQGVGY vNELIARLTg	q.AVRDETqT	NRTLdSDPAT FPLNrTFYAD
<i>A. terreus</i> 9a1	PLGPvQGVGY aNELMARLTR	A.PVHDHTCv	NNTLDASPAT FPLNATLYAD
<i>A. terreus</i> cbs	PLGPvQGVGY aNELIARLTR	S.PVHDHTCv	NNTLDANPAT FPLNATLYAD
<i>A. niger</i> var. <i>awamori</i>	PLGPTQGVGY aNELIARLTa	S.PVHDDTSS	NHTLdSNPAT FPLNSTLYAD
<i>A. niger</i> T213	PLGPTQGVGY aNELIARLTa	S.PVHDDTSS	NHTLdSNPAT FPLNSTLYAD
<i>A. niger</i> NRRL3135	PLGPTQGVGY aNELIARLTa	S.PVHDDTSS	NHTLdSSPAT FPLNSTLYAD
<i>A. fumigatus</i> ATCC13073	PLGPAQGIGF tNELIARLTR	S.PVQDHTST	NeTLvSNPAT FPLNATMYvD
<i>A. fumigatus</i> ATCC32722	PLGPAQGIGF tNELIARLTR	S.PVQDHTST	NeTLvSNPAT FPLNATMYvD
<i>A. fumigatus</i> ATCC58128	PLGPAQGIGF tNELIARLTR	S.PVQDHTST	NeTLvSNPAT FPLNATMYvD
<i>A. fumigatus</i> ATCC26906	PLGPAQGIGF tNELIARLTR	S.PVQDHTST	NeTLvSNPAT FPLNATMYvD
<i>A. fumigatus</i> ATCC32239	PLGPAQGIGF tNELIARLTN	S.PVQDHTST	NeTLdSDPAT FPLNATIYvD
<i>E. nidulans</i>	PLGPAQGIGF tNELIARLTQ	S.PVQDNTST	NHTLdSNPAT FPLDrkLYAD
<i>T. thermophilus</i>	PLGPAQGVGF vNELIARMTg	S.PVQDYTTv	NHTLdSNPAT FPLNATLYAD
<i>T. lanuginosa</i>	AFGPSRGVGF vNELIARMTg	NLPVKDHTTv	NHTLDdNPET FPLDAvLYAD
<i>M. thermophila</i>	PLGPTQGVGF vNELIARLA	GvPVRDgTST	NRTLdGDPrt FPLGrPLYAD

Consensus Seq. 11

PLGPAQGVGF -NELIARLTa S-PVQDHTST NHTLdSNPAT FPLNATLYAD

	351		400
<i>P. involutus</i> (phyA1)	FSHDNlMVAV FsAMGLFrqP	aPLSTSVpNP	wrt.....Wr TSSlVPFSGR
<i>P. involutus</i> (phyA2)	FSHDNlMVAV FsAMGLFrqS	aPLSTSTpDP	nrt.....Wl TSSvVPFSAR
<i>T. pubescens</i>	FSHDNqMVAI FsAMGLFNqS	aPLdPTTpDP	art.....Fl vkkivPFsAR
<i>A. pediades</i>	LSHDNqMIAI FsAMGLFNqS	sPLdPSfpNP	krt.....Wv TSrltPFsAR
<i>P. lycii</i>	FSHDNTMVPI FaALGLFNAT	a.LdPlkpDe	nrl.....Wv DSkivPFSGH
<i>A. terreus</i> 9a1	FSHDSnLVSI FWALGLYNGT	aPLSqtSVES	Vs..QTDGYA AAWTVPFAAR
<i>A. terreus</i> cbs	FSHDSnLVSI FWALGLYNGT	KPLSqtTVEd	It..rTDGYA AAWTVPFAAR
<i>A. niger</i> var. <i>awamori</i>	FSHDNGIISI LFALGLYNGT	KPLSTTTVEN	It..QTDGFS SAWTVPFASR
<i>A. niger</i> T213	FSHDNGIISI LFALGLYNGT	KPLSTTTVEN	It..QTDGFS SAWTVPFASR
<i>A. niger</i> NRRL3135	FSHDNGIISI LFALGLYNGT	KPLSTTTVEN	It..QTDGFS SAWTVPFASR
<i>A. fumigatus</i> ATCC13073	FSHDNSMVSI FFALGLYNGT	EPLSrTSVES	ak..ElDGYS ASWvVPFGAR
<i>A. fumigatus</i> ATCC32722	FSHDNSMVSI FFALGLYNGT	gPLSrTSVES	ak..ElDGYS ASWvVPFGAR
<i>A. fumigatus</i> ATCC58128	FSHDNSMVSI FFALGLYNGT	EPLSrTSVES	ak..ElDGYS ASWvVPFGAR
<i>A. fumigatus</i> ATCC26906	FSHDNSMVSI FFALGLYNGT	EPLSrTSVES	ak..ElDGYS ASWvVPFGAR
<i>A. fumigatus</i> ATCC32239	FSHDNGMIPI FFAMGLYNGT	EPLSqtSeES	tk..ESNGYS ASWAVPFGAR
<i>E. nidulans</i>	FSHDNSMISI FFAMGLYNGT	QPLSmdSVES	Iq..EmDGYA ASWTVPFGAR
<i>T. thermophilus</i>	FSHDNTMtSI FaALGLYNGT	akLSTTeIKS	Ie..ETDGYS AAWTVPFGGR
<i>T. lanuginosa</i>	FSHDNTMtGI FsAMGLYNGT	KPLSTSkIQP	ptgaAADGYA ASWTVPFAAR
<i>M. thermophila</i>	FSHDNdMMGV LgALGaYDgV	pPLdkTArrd	..peElGGYA ASWAVPFAAR

Consensus Seq. 11

FSHDNTMVSI FFALGLYNGT KPLSTTSVES I---ETDGYA ASWTVPFAAR

	401		450
<i>P. involutus</i> (phyA1)	mvVERLsC.. fGt.....Tk	VRVLVQDQVq PLEfCGgDRn
<i>P. involutus</i> (phyA2)	maVERLsC.. AGt.....Tk	VRVLVQDQVq PLEfCGgDQd
<i>T. pubescens</i>	mvVERLDC.. GGa.....Qs	VRLLVNDaVq PLafCGaDts
<i>A. pediades</i>	mvtErLlCQr DGtGsGGpsr	imrNgnvQTF	VRILVNDaLq PLkfCGgDmd
<i>P. lycii</i>	mtVEkLaC..sgKea	VRVLVNDaVq PLEfCGg.vd
<i>A. terreus</i> 9a1	AYVEMMQCrAEK...EPL	VRVLVNDVRM PLHGCPtDKL
<i>A. terreus</i> cbs	AYIEMMQCrAEK...QPL	VRVLVNDVRM PLHGCAVDNL
<i>A. niger</i> var. <i>awamori</i>	LYVEMMQCQAEQ...EPL	VRVLVNDRVV PLHGCPIDaL
<i>A. niger</i> T213	LYVEMMQCQAEQ...EPL	VRVLVNDRVV PLHGCPIDaL
<i>A. niger</i> NRRL3135	LYVEMMQCQAEQ...EPL	VRVLVNDRVV PLHGCPVDaL
<i>A. fumigatus</i> ATCC13073	AYfEtMQCKsEK...EPL	VraLINDRVV PLHGCDVDKL
<i>A. fumigatus</i> ATCC32722	AYfEtMQCKsEK...EPL	VraLINDRVV PLHGCDVDKL
<i>A. fumigatus</i> ATCC58128	AYfEtMQCKsEK...ESL	VraLINDRVV PLHGCDVDKL
<i>A. fumigatus</i> ATCC26906	AYfEtMQCKsEK...EPL	VraLINDRVV PLHGCDVDKL
<i>A. fumigatus</i> ATCC32239	AYfEtMQCKsEK...EPL	VraLINDRVV PLHGCAVDKL
<i>E. nidulans</i>	AYfELMQCE.KK...EPL	VRVLVNDRVV PLHGCEVDsL
<i>T. thermophilus</i>	AYIEMMQCDDsD...EPV	VRVLVNDRVV PLHGCEVDsL
<i>T. lanuginosa</i>	AYVELLRcET ETsSeEEeEG	..ED...EPF	VRVLVNDRVV PLHGCrVDRW
<i>M. thermophila</i>	iYVEkMRCsG GGgGgGGgEG	..rQekdEeM	VRVLVNDVRM TLkGCGaDer

Consensus Seq. 11 AYVEMMQCEA GG-G-GG-EG --EK---EPL VRVLVNDRVV PLHGCGVDKL

	451		482
<i>P. involutus</i> (phyA1)	GLCtLAKFVE SqTFARSDga	GDFEKCFAts a~	
<i>P. involutus</i> (phyA2)	GLCaLDKFVE SqAYARSGga	GDFEKCLAtt v~	
<i>T. pubescens</i>	GvCtLDAFVE SqAYARNDge	GDFEKCFat~ --	
<i>A. pediades</i>	SLCtLEAFVE SqkYAReDgq	GDFEKCFD-- --	
<i>P. lycii</i>	GvCELSAFVE SqTYARENgq	GDFAKCGfvp se	
<i>A. terreus</i> 9a1	GRCKrDAFVA GLSFAQAG..	GNWADCF--- --	
<i>A. terreus</i> cbs	GRCKrDDFVE GLSFARAG..	GNWAECF--- --	
<i>A. niger</i> var. <i>awamori</i>	GRCtrDsFVr GLSFARSG..	GDWAECsA-- --	
<i>A. niger</i> T213	GRCtrDsFVr GLSFARSG..	GDWAECFA-- --	
<i>A. niger</i> NRRL3135	GRCtrDsFVr GLSFARSG..	GDWAECFA-- --	
<i>A. fumigatus</i> ATCC13073	GRCKLNDFVK GLSWARSG..	GNWGECFS-- --	
<i>A. fumigatus</i> ATCC32722	GRCKLNDFVK GLSWARSG..	GNWGECFS-- --	
<i>A. fumigatus</i> ATCC58128	GRCKLNDFVK GLSWARSG..	GNWGECFS-- --	
<i>A. fumigatus</i> ATCC26906	GRCKLNDFVK GLSWARSG..	GNWGECFS-- --	
<i>A. fumigatus</i> ATCC32239	GRCKLKDFVK GLSWARSG..	GNSEQSFS-- --	
<i>E. nidulans</i>	GRCKrLDDWVE GLNFARSG..	GNWktCFTl-	
<i>T. thermophilus</i>	GRCKrDDFVr GLSFARqG..	GNWEGCYAas e-	
<i>T. lanuginosa</i>	GRCKrDEWIK GLTFARqG..	GHWDrcF--- --	
<i>M. thermophila</i>	GmCtLErFIE SMAFARGN..	GKWDlCFA-- --	

Consensus Seq. 11 GRCKLDDFVE GLSFARSG-- GNWAECFA-- --

Figure 7

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M G V F V V L L S I A T L F G S T S G T      20
ATGGGCGTGTTCGTGCTACTGTCCATTGCCACCTTGTTTCGGTTCCACATCCGGTACC
1 -----+-----+-----+-----+-----+-----+-----+ 60
TACCCGCAACAAGCAGCAGCATGACAGGTAACGGTGGAACAAGCCAAGGTGTAGGCCATGG

A L G P R G N S H S C D T V D G G Y Q C      40
GCCTTGGGTCTCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT
61 -----+-----+-----+-----+-----+-----+-----+ 120
CGGAACCCAGGAGCACCATTAAAGAGTGAGAACACTGTGACAACTGCCACCAATGGTTACA

F P E I S H L W G T Y S P Y F S L A D E      60
TTCCCAGAAATTTCTCACTTGTGGGGTACCTACTCTCCATACTTCTCTTTGGCAGACGAA
121 -----+-----+-----+-----+-----+-----+-----+ 180
AAGGGTCTTTAAAGAGTGAACACCCCATGGATGAGAGGTATGAAGAGAAACCGTCTGCTT

S A I S P D V P D D C R V T F V Q V L S      80
TCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTTCGTTCAAGTTTTGTCT
187 -----+-----+-----+-----+-----+-----+-----+ 240
AGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAGTTCAAAACAGA

R H G A R Y P T S S A S K A Y S A L I E      100
AGACACGGTGCTAGATACCCAATTCTTCTGCGTCTAAGGCTTACTCTGCTTTGATTGAA
241 -----+-----+-----+-----+-----+-----+-----+ 300
TCTGTGCCACGATCTATGGGTTGAAGAAGACGCAGATTCCGAATGAGACGAAACTAATT

A I Q K N A T A F K G K Y A F L K T Y N      120
GCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAC
301 -----+-----+-----+-----+-----+-----+-----+ 360
CGATAAGTTTCTTGCATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTG

Y T L G A D D L T P F G E N Q M V N S G      140
TACACTTTGGGTGCTGACGACTTGACTCCATTTCGGTGAAAACCAAATGGTTAACTCTGGT
361 -----+-----+-----+-----+-----+-----+-----+ 420
ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACCAATTGAGACCA

I K F Y R R Y K A L A R K I V P F I R A      160
ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTTCATTAGAGCT
421 -----+-----+-----+-----+-----+-----+-----+ 480
TAATTCAGATGTCCTTCTATGTTCCGAAACCGATCTTCTAACAAGGTAAGTAATCTCGA

S G S D R V I A S A E K F I E G F Q S A      180
TCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTTTCCAATCTGCT
481 -----+-----+-----+-----+-----+-----+-----+ 540
AGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAAAGGTTAGACGA

K L A D P G S Q P H Q A S P V I N V I I      200
AAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTAACGTGATCATT
541 -----+-----+-----+-----+-----+-----+-----+ 600
TTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTTCGAAGAGGTCAATAATGCACTAGTAA

P E G S G Y N N T L D H G T C T A F E D      220
CCAGAAGGATCCGGTTACAACAACACTTTGGACCACGGTACTTGTACTGCTTTCGAAGAC
601 -----+-----+-----+-----+-----+-----+-----+ 660
GGTCTTCCTAGGCCAATGTTGTTGTGAAACCTGGTGCCATGAACATGACGAAAGCTTCTG

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S E L G D D V E A N F T A L F A P A I R 240
 TCTGAATTAGGTGACGACGTTGAAGCTAACTTCACTGCTTTGTTTCGCTCCAGCTATTAGA
 661 -----+-----+-----+-----+-----+ 720
 AGACTTAATCCACTGCTGCAACTTCGATTGAAGTGACGAAACAAGCGAGGTGATAATCT

 A R L E A D L P G V T L T D E D V V Y L 260
 GCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACGAAGACGTTGTTTACTTG
 721 -----+-----+-----+-----+-----+ 780
 CGATCTAACCTTCGACTGAACGGTCCACAATGAACTGACTGCTTCTGCAACAAATGAAC

 M D M C P F D T V A R T S D A T E L S P 280
 ATGGACATGTGTCCATTGACACTGTCGCTAGAACTTCTGACGCTACTGAATTGTCTCCA
 781 -----+-----+-----+-----+-----+ 840
 TACCTGTACACAGGTAAGCTGTGACAGCGATCTTGAAGACTGCGATGACTTAACAGAGGT

 F C A L F T H D E W I Q Y D Y L Q S L G 300
 TTCTGTGCTTTGTTCACTCACGACGAATGGATCCAATACGACTACTTGCAAAGCTTGGGT
 841 -----+-----+-----+-----+-----+ 900
 AAGACACGAAACAAGTGAGTGCTGCTTACCTAGGTTATGCTGATGAACGTTTCGAACCCA

 K Y Y G Y G A G N P L G P A Q G V G F A 320
 AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGCT
 901 -----+-----+-----+-----+-----+ 960
 TTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTCGAGTTCACAACCAAAGCGA

 N E L I A R L T H S P V Q D H T S T N H 340
 AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC
 961 -----+-----+-----+-----+-----+ 1020
 TTGCTTAACCTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG

 T L D S N P A T F P L N A T L Y A D F S 360
 ACTTTGGACTCTAACCAGCTACTTTCCCATTTGAACGCTACTTTGTACGCTGACTTCTCT
 1021 -----+-----+-----+-----+-----+ 1080
 TGAAACCTGAGATTGGGTGCGATGAAAGGGTAACCTGCGATGAAACATGCGACTGAAGAGA

 H D N T M I S I F F A L G L Y N G T K P 380
 CACGACAACACTATGATATCTATTTTCTTCGCTTTGGGTTGTACAACGGTACCAAGCCA
 1081 -----+-----+-----+-----+-----+ 1140
 GTGCTGTTGTGATACTATAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGTTTCGGT

 L S T T S V E S I E E T D G Y S A S W T 400
 TTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTTACTCTGCTTCTTGGACT
 1141 -----+-----+-----+-----+-----+ 1200
 AACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA

 V P F A A R A Y V E M M Q C Q A E K E P 420
 GTTCCATTGCTGCTAGAGCTTACGTTGAAATGATGCAATGTCAAGCTGAAAAGGAACCA
 1201 -----+-----+-----+-----+-----+ 1260
 CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACAGTTCGACTTTTCCTTGGT

 L V R V L V N D R V V P L H G C A V D K 440
 TTGGTTAGAGTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTGCTGTTGACAAG
 1261 -----+-----+-----+-----+-----+ 1320
 AACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACGACAACCTGTT

L G R C K R D D F V E G L S F A R S G G 460
TTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTAGATCTGGTGGT
1321 -----+-----+-----+-----+-----+ 1380
AACCCTCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA

N W A E C F A * 467
AACTGGGCTGAATGTTTCGCTTAA
1381 -----+-----+----- 1410
TTGACCCGACTTACAAAGCGAATT

Figure 8

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M G V F V V L L S I A T L F G S T S G T      20
ATGGGCGTGTTCGTCTGCTACTGTCCATTGCCACCTTGTTCGGTTCACATCCGGTACC
1  -----+-----+-----+-----+-----+ 60
TACCCGCAAGCAGCAGCATGACAGGTAACGGTGGAACAAGCCAAGGTGTAGGCCATGG

A L G P R G N S H S C D T V D G G Y Q C      40
GCCTTGGGTCTCGTGGTAACTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT
61 -----+-----+-----+-----+-----+ 120
CGGAACCCAGGAGCACCATTGAGAGTGAGAACACTGTGACAACTGCCACCAATGGTTACA

F P E I S H L W G T Y S P F F S L A D E      60
TTCCCAGAAATTTCTCACTTGTGGGGTACATACTCTCCATTCTTCTCTTTGGCTGACGAA
121 -----+-----+-----+-----+-----+ 180
AAGGGTCTTTAAAGAGTGAACACCCCATGTATGAGAGGTAAGAAGAGAAACCGACTGCTT

S A I S P D V P K G C R V T F V Q V L S      80
TCTGCTATTTCTCCAGACGTTCCAAAGGGTGTAGAGTTACTTTCGTTCAAGTTTGTCT
181 -----+-----+-----+-----+-----+ 240
AGACGATAAAGAGGTCTGCAAGGTTTCCCAACATCTCAATGAAAGCAAGTTCAAAACAGA

R H G A R Y P T S S A S K A Y S A L I E      100
AGACACGGTGCTAGATACCAACTTCTTCTGCGTCTAAGGCGTACTCTGCTTTGATTGAA
241 -----+-----+-----+-----+-----+ 300
TCTGTGCCACGATCTATGGGTGAAGAAGACGCAGATTCCGCATGAGACGAAACTAATT

A I Q K N A T A F K G K Y A F L K T Y N      120
GCTATTCAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTCTTGAAGACTTACAAC
301 -----+-----+-----+-----+-----+ 360
CGATAAGTTTTCTTGCATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTG

Y T L G A D D L T P F G E Q Q M V N S G      140
TACACTTTGGGTGCTGACGACTTGACTCCATTCCGGTGAACAACAAATGGTTAACTCTGGT
361 -----+-----+-----+-----+-----+ 420
ATGTGAAACCCACGACTGCTGAAGTGAAGGTAAGCCACTTGTGTTTACCAATTGAGACCA

I K F Y R R Y K A L A R K I V P F I R A      160
ATTAAGTTCTACAGAAGATAAAGGCTTTGGCTAGAAAGATTGTTCCATTCAATTAGAGCT
421 -----+-----+-----+-----+-----+ 480
TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTAAGTAATCTCGA

S G S D R V I A S A E K F I E G F Q S A      180
TCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTTCAATGAAGGTTTCCAATCTGCT
481 -----+-----+-----+-----+-----+ 540
AGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAAAGGTTAGACGA

K L A D P G A N P H Q A S P V I N V I I      200
AAGTTGGCTGACCCAGGTGCTAACCACACCAAGCTTCTCCAGTTATTAACGTTATTATT
541 -----+-----+-----+-----+-----+ 600
TTCAACCGACTGGGTCCACGATTGGGTGTGGTTCGAAGAGGTCAATAATTGCAATAATAA

P E G A G Y N N T L D H G L C T A F E E      220
CCAGAAGGTGCTGGTTACAACAACACTTTGGACCACGGTTTGTGTACTGCTTTCGAAGAA
601 -----+-----+-----+-----+-----+ 660
GGTCTTCCACGACCAATGTTGTTGTGAAACCTGGTGCCAAACACATGACGAAAGCTTCTT

```

S E L G D D V E A N F T A V F A P P I R 240
 TCTGAATTGGGTGACGACGTTGAAGCTAACTTCACTGCTGTTTTCGCTCCACCAATTAGA
 661 -----+-----+-----+-----+-----+ 720
 AGACTTAACCCACTGCTGCAACTTCGATTGAAGTGACGACAAAAGCGAGGTGGTTAATCT

 A R L E A H L P G V N L T D E D V V N L 260
 GCTAGATTGGAAGCTCACTTGCCAGGTGTTAACTTGAAGTACGACGACGTTGTTAACTTG
 721 -----+-----+-----+-----+-----+ 780
 CGATCTAACCTTCGAGTGAACGGTCCACAATTGAAGTGAAGTCTGCTTCTGCAACAATTGAAC

 M D M C P F D T V A R T S D A T Q L S P 280
 ATGGACATGTGTCCATTTCGACACTGTTGCTAGAACTTCTGACGCTACTCAATTGTCTCCA
 781 -----+-----+-----+-----+-----+ 840
 TACCTGTACACAGGTAAGCTGTGACAACGATCTTGAAGACTGCGATGAGTTAACAGAGGT

 F C D L F T H D E W I Q Y D Y L Q S L G 300
 TTCTGTGACTTGTTCACCTCACGACGAATGGATTCAATACGACTACTTGCAATCTTTGGGT
 841 -----+-----+-----+-----+-----+ 900
 AAGACACTGAACAAGTGAGTGTGCTTACCTAAGTTATGCTGATGAACGTTAGAAACCCA

 K Y Y G Y G A G N P L G P A Q G V G F V 320
 AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGTT
 901 -----+-----+-----+-----+-----+ 960
 TTCATGATGCCAATGCCACGACCATTTGGGTAACCCAGGTGAGTTCCACAACCAAAGCAA

 N E L I A R L T H S P V Q D H T S T N H 340
 AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC
 961 -----+-----+-----+-----+-----+ 1020
 TTGCTTAACCTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG

 T L D S N P A T F P L N A T L Y A D F S 360
 ACTTTGGACTCTAACCAGCTACTTTCCCATTTGAACGCTACTTTGTACGCTGACTTCTCT
 1021 -----+-----+-----+-----+-----+ 1080
 TGAAACCTGAGATTGGGTGATGAAAGGGTAACCTTCCGATGAAACATGCGACTGAAGAGA

 H D N T M V S I F F A L G L Y N G T K P 380
 CACGACAACACTATGGTTTCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACTAAGCCA
 1081 -----+-----+-----+-----+-----+ 1140
 GTGCTGTTGTGATACCAAAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGATTCCGT

 L S T T S V E S I E E T D G Y S A S W T 400
 TTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTTACTCTGCTTCTTGGACT
 1141 -----+-----+-----+-----+-----+ 1200
 AACAGATGATGAAGACAACCTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA

 V P F A A R A Y V E M M Q C E A E K E P 420
 GTTCCATTGCTGCTAGAGCTTACGTTGAAATGATGCAATGTGAAGCTGAAAAGGAACCA
 1201 -----+-----+-----+-----+-----+ 1260
 CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACACTTCGACTTTTCCTTGGT

 L V R V L V N D R V V P L H G C G V D K 440
 TTGGTTAGAGTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTTGGTGTGACAAG
 1261 -----+-----+-----+-----+-----+ 1320
 AACCAATCTCAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACCACAACCTGTTT

L G R C K R D D F V E G L S F A R S G G 460
TTGGGTTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTAGATCTGGTGGT
1321 -----+-----+-----+-----+-----+ 1380
AACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA

N W E E C F A * 467
AACTGGGAAGAATGTTTCGCTTAA
1381 -----+-----+-----+-----+ 1404
TTGACCCTTCTTACAAAGCGAATT

Figure 9

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M G V F V V L L S I A T L F G S T S G T 20
ATGGGGGTTTTTCGTCGTTCTATTATCTATCGCGACTCTGTTCCGGCAGCACATCGGGCACT
1 -----+-----+-----+-----+-----+ 60
TACCCCCAAAAGCAGCAAGATAATAGATAGCGCTGAGACAAGCCGTCGTGTAGCCCCGTGA

A L G P R G N H S K S C D T V D L G Y Q 40
GCGCTGGGCCCCCGTGGAAATCACTCCAAGTCCTGCGATACGGTAGACCTAGGGTACCAG
61 -----+-----+-----+-----+-----+ 120
CGCGACCCGGGGGCACCTTTAGTGAGGTTTCAGGACGCTATGCCATCTGGATCCCATGGTC

C S P A T S H L W G T Y S P Y F S L E D 60
TGCTCCCCCTGCGACTTCTCATCTATGGGGCAGTACTCGCCATaCTTTTCGCTCGAGGAC
121 -----+-----+-----+-----+-----+ 180
ACGAGGGGACGCTGAAGAGTAGATACCCCGtgCATGAGCGGTatGAAAAGCGAGCTCCTG

E L S V S S K L P K D C R I T L V Q V L 80
GAGCTGTCCGTGTCGAGTAAGCTTCCCAAGGATTGCCGGATCACCTTGGTACAGGTGCTA
181 -----+-----+-----+-----+-----+ 240
CTCGACAGGCACAGCTCATTTCGAAGGGTTCCTAACGGCCTAGTGGAACCATGTCCACGAT

S R H G A R Y P T S S K S K K Y K K L I 100
TCGCGCCATGGAGCGCGGTACCCAACCAGCTCCAAGAGCAAAAAGTATAAGAAGCTTaTt
241 -----+-----+-----+-----+-----+ 300
AGCGCGGTACCTCGCGCCATGGGTTGGTTCGAGGTTCTCGTTTTTCATATTCTTCGAAtAa

T A I Q A N A T D F K G K Y A F L K T Y 120
ACGGCGATCCAGGCCAATGCCACCGACTTCAAGGGCAAGTAcGCCTTTTTGAAGACGTAC
301 -----+-----+-----+-----+-----+ 360
TGCCGCTAGGTCCGGTTACGGTGGCTGAAGTTCCTCGTTCatgCGGAAAACTTCTGCATG

N Y T L G A D D L T P F G E Q Q L V N S 140
AACTATACTCTGGGTGCGGATGACCTCACTCCCTTTGGGGAGCAGCAGCTGGTGAACCTCG
361 -----+-----+-----+-----+-----+ 420
TTGATATGAGACCCACGCCTACTGGAGTGAGGGAAACCCCTCGTCGTCGACCACTTGAGC

G I K F Y Q R Y K A L A R S V V P F I R 160
GGCATCAAGTTCTACCAGAGGTACAAGGCTCTGGCGCGCAGTGTGGTGCCGTTTATTTCGC
421 -----+-----+-----+-----+-----+ 480
CCGTAGTTCAAGATGGTCTCCATGTTCCGAGACCGCGCGTCACACCACGGCAAATAAGCG

A S G S D R V I A S G E K F I E G F Q Q 180
GCCTCAGGCTCGGACCGGGTTATTGCTTCGGGAGAGAAGTTTCATCGAGGGGTTCCAGCAG
481 -----+-----+-----+-----+-----+ 540
CGGAGTCCGAGCCTGGCCCCAATAACGAAGCCCTCTCTTCAAGTAGCTCCCCAAGGTCGTC

A K L A D P G A T N R A A P A I S V I I 200
GCGAAGCTGGCTGATCCTGGCGCGACGAACCGCGCGCTCCGGCGATTAGTGTGATTATT
541 -----+-----+-----+-----+-----+ 600
CGCTTCGACCGACTAGGACCGCGCTGCTTGGCGCGGCGAGGCCGCTAATCACACTAATAA

P E S E T F N N T L D H G V C T K F E A 220
CCGGAGAGCGGAGACGTTCAACAATACGCTGGACCACGGTGTGTGCACGAAGTTTGAGGCG
601 -----+-----+-----+-----+-----+ 660
GGCCTCTCGCTCTGCAAGTTGTTATGCGACCTGGTGCCACACACGTGCTTCAAACCTCCGC

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S Q L G D E V A A N F T A L F A P D I R 240
AGTCAGCTGGGAGATGAGGTTCGCGCCAATTTCACTGCGCTCTTGCACCCGACATCCGA 720
-----+-----+-----+-----+-----+
TCAGTCGACCCTCTACTCCAACGCCGTTAAAGTGACGCGAGA AACGTGGGCTGTAGGCT

A R L E K H L P G V T L T D E D V V S L 260
GCTCGCctCGAGAAGCATCTTCCTGGCGTGACGCTGACAGACGAGGACGTTGT CAGTCTA 780
-----+-----+-----+-----+-----+
CGAGCGgaGCTCTTCGTAGAAGGACCGCACTGCGACTGTCTGCTCCTGCAACAGTCAGAT

M D M C P F D T V A R T S D A S Q L S P 280
ATGGACATGTGTcCGTTTTGATACGGTAGCGCGCACCCAGCGACGCAAGTCAGCTGT CACCG 840
-----+-----+-----+-----+-----+
TACCTGTACACagGCAA ACTATGCCATCGCGCGTGGTTCGCTGCGTT CAGTCGACAGTGGC

F C Q L F T H N E W K K Y D Y L Q S L G 300
TTCTGTCAACTCTTCACTCACAATGAGTGGAAGAAGTACgACTACCTTCAGTCCTTGGGC 900
-----+-----+-----+-----+-----+
AAGACAGTTGAGAAGTGAGTGTTACTCACCTTCTT CATGcTGATGGAAGTCAGGAACCCG

K Y Y G Y G A G N P L G P A Q G I G F T 320
AAGTACTACGGCTACGGCGCAGGCAACCCCTCTGGGACCGGCTCAGGGGATAGGGTTCACC 960
-----+-----+-----+-----+-----+
TTCATGATGCCGATGCCGCGTCCGTTGGGAGACCCCTGGCCGAGTCCCCTATCCCAAGTGG

N E L I A R L T R S P V Q D H T S T N S 340
AACGAGCTGATTGCCCCGTTGACgCGTTCGCCAGTG CAGGACCACACCAGCACTAACTCG 1020
-----+-----+-----+-----+-----+
TTGCTCGACTAACGGGCCAACTGcGCAAGCGGTCA CGT CCTGGTGTGGTTCGTGATTGAGC

T L V S N P A T F P L N A T M Y V D F S 360
ACTCTAGTCTCCAACCCGGCCACCTTCCCGTTGAACGCTACCATGTACGTCGACTTTTCA 1080
-----+-----+-----+-----+-----+
TGAGATCAGAGGTTGGGCCGGTGGAAGGGCAACTT GCGATGGTACATGCAGCTGAAAAGT

H D N S M V S I F F A L G L Y N G T E P 380
CACGACAACAGCATGGTTTCCATCTTCTTGCATTGGGCCTGTACAACGGCACTGAACCC 1140
-----+-----+-----+-----+-----+
GTGCTGTTGTCTACCAAAGGTAGAAGAAACGTA ACCCGGACATGTTGCCGTGACTTGGG

L S R T S V E S A K E L D G Y S A S W V 400
TTGTCCCGGACCTCGGTGGAAGCGCCAAGGAATTGGATGGGTATTCTGCATCCTGGGTG 1200
-----+-----+-----+-----+-----+
AACAGGGCCTGGAGCCACCTTTCGCGGTTCTTA ACCTACCATAAGACGTAGGACCCAC

V P F G A R A Y F E T M Q C K S E K E P 420
GTGCCCTTTCGGCGCGCGAGCCTACTTCGAGACGATGCAATGCAAGTCGGA AAAAGGAGCCT 1260
-----+-----+-----+-----+-----+
CACGGAAAGCCGCGCGCTCGGATGAAGCTCTGCTAC GTTACGTTACGCTTCAGCCTTTTCTCGGA

L V R A L I N D R V V P L H G C D V D K 440
CTTGTTTCGCGCTTTGATTAATGACCGGTTGTGCCACTGCATGGCTGCGATGTGGACAAG 1320
-----+-----+-----+-----+-----+
GAACAAGCGCGAAACTAATTACTGGCCCAACCGGTGACGTACCGACGCTACACCTGTTC

L G R C K L N D F V K G L S W A R S G G 460
CTGGGGCGATGCAAGCTGAATGACTTTGTCAAGGGATTGAGTTGGGCCAGATCTGGGGGC
1321 -----+-----+-----+-----+-----+ 1380
GACCCCGCTACGTTGACTTACTGAAACAGTTCCCTAACTCAACCCGGTCTAGACCCCCG

N W G E C F S * 467
AACTGGGGAGAGTGCTTTAGTTGA
1381 -----+-----+-----+-----+ 1404
TTGACCCCTCTCACGAAATCAACT

Figure 10

CP-1

Eco RI M G V F V V L L S I A T L F G S T
 TATATGAATTCATGGGCGTGTTCGTCTGCTACTGTCCATTGCCACCTTGTTCCGGTTCCA
 1 -----+-----+-----+-----+-----+ 60
 ATATACTTAAGTACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGT

S G T A L G P R G N S H S C D T V D G G
 CATCCGGTACCGCCTTGGGTCCTCGTGTAATTCTCACTCTTGACACTGTTGACGGTG
 61 -----+-----+-----+-----+-----+ 120
 GTAGGCCATGGCGGAACCCAGGAGCACCATTAAAGAGTGAGAACAACCTGTGACAACCTGCCAC

CP-2

CP-3

Y Q C F P E I S H L W G Q Y S P Y F S L
 GTTACCAATGTTTCCCAGAAATTTCTCACTTGTGGGGTCAATACTCTCCATACTTCTCTT
 121 -----+-----+-----+-----+-----+ 180
 CAATGGTTACAAAGGGTCTTTAAAGAGTGAACACCCAGTTATGAGAGGTATGAAGAGAA

E D E S A I S P D V P D D C R V T F V Q
 TGGAGACGAATCTGCTATTTCTCCAGACGTTCAGACGACTGTAGAGTTACTTTCGTTCT
 181 -----+-----+-----+-----+-----+ 240
 ACCTTCTGCTTAGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAG

CP-4.7

CP-5.7

V L S R H G A R Y P T D S K G K K Y S A
 AAGTTTTGTCTAGACACGGTGCTAGATACCCAAGTgacTCTAAGggtAAGaagTACTCTG
 241 -----+-----+-----+-----+-----+ 300
 TTCAAACAGATCTGTGCCACGATCTATGGGTTGActgAGATTCCcaTTcttCATGAGAC

L I E A I Q K N A T A F K G K Y A F L K
 CTTTGATTGAAGCTATTCAAAGAAGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGA
 301 -----+-----+-----+-----+-----+ 360
 GAAACTAAGTTCGATAAGTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAAGT

CP-6

CP-7

T Y N Y T L G A D D L T P F G E N Q M V
 AGACTTACAACCTACACTTTGGGTGCTGACGACTTGACTCCATTGCGGTGAAAACCAAATGG
 361 -----+-----+-----+-----+-----+ 420
 TCTGAATGTTGATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACC

N S G I K F Y R R Y K A L A R K I V P F
 TTAAGTCTGGTATTAAAGTTCTACAGAAGATAAAGGCTTTGGCTAGAAAGATTGTTCCAT
 421 -----+-----+-----+-----+-----+ 480
 AATTGAGACCATAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTA

CP-8.7

CP-9

I R A S G S S R V I A S A E K F I E G F
 TCATTAGAGCTTCTGGTTCTtctAGAGTTATTGCTTCTGCTGAAAAGTTTCAAGGTTT
 481 -----+-----+-----+-----+-----+ 540
 AGTAATCTCGAAGACCAAGaagTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAA

Q S A K L A D P G S Q P H Q A S P V I D
 TCCAATCTGCTAAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTG
 541 -----+-----+-----+-----+-----+ 600
 AGGTTAGACGATTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTTCAAGAGGTCAATAAC

CP-10.7CP-11.7

V I I S E A S S Y N N T L D P G T C T A
 ACGTTATTATTtctGAcgctTCTtctTACAACAACACTTTGGACccaGGTACTTGTACTG
 601 -----+-----+-----+-----+-----+ 660
 TGCAATAATAAagaCTgcgaAGGagaATGTTGTTGTGAAACCTGggtCCATGAACATGAC

F E D S E L A D T V E A N F T A L F A P
 CTTTCGAAGACTCTGAATTGgctGACactGTTGAAGCTAACTTCACTGCTTTGTTGCTC
 661 -----+-----+-----+-----+-----+ 720
 GAAAGCTTCTGAGACTTAACcgaCTGtgaCAACTTCGATTGAAGTGACGAAACAAGCGAG

CP-12.7

A I R A R L E A D L P G V T L T D T E V
 CAGCTATTAGAGCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACactgaaG
 721 -----+-----+-----+-----+-----+ 780
 GTCGATAATCTCGATCTAACCTTCGACTGAACGGTCCACAATGAACTGACTGtgacttC

CP-13.7

T Y L M D M C S F E T V A R T S D A T E
 TTactTACTTGATGGACATGTGTtctTTTCGAAACTGTTGCTAGAACTTCTGACGCTACTG
 781 -----+-----+-----+-----+-----+ 840
 AAtgaATGAACTACCTGTACACAagaAAGCTTTGACAACGATCTTGAAGACTGCGATGAC

L S P F C A L F T H D E W R H Y D Y L Q
 AATTGTCTCCATTCTGTGCTTTGTTCACTCACGACGAATGGAGAcactACGACTACTTGC
 841 -----+-----+-----+-----+-----+ 900
 TTAACAGAGGTAAGACACGAAACAAGTGAGTGCTGCTTACCTCTgtgATGCTGATGAACG

CP-14.7CP-15.7

S L K K Y Y G H G A G N P L G P T Q G V
 AATCTTTGaagAAGTACTACGGTcacGGTGCTGGTAACCCATTGGGTCCAactCAAGGTG
 901 -----+-----+-----+-----+-----+ 960
 TTAGAAACTtctTTCATGATGCCAgTgCCACGACCATTGGGTAAACCAGGtgaGTTCCAC

G F A N E L I A R L T R S P V Q D H T S
 TTGGTTTCGCTAACGAATTGATTGCTAGATTGACTAGATCTCCAGTTCAAGACCACACTT
 961 -----+-----+-----+-----+-----+ 1020
 AACCAAAGCGATTGCTTAACTAACGATCTAACTGATCTAGAGGTCAAGTTCTGGTGTGAA

CP-16

CP-17.7

T N H T L D S N P A T F P L N A T L Y A
 CTACTAACCCACACTTTGGACTCTAACCCAGCTACTTTCCCATTTGAACGCTACTTTGTACG
 1021 -----+-----+-----+-----+-----+ 1080
 GATGATTGGTGTGAAACCTGAGATTGGGTGCGATGAAAGGGTAACCTTGCATGAAACATGC

D F S H D N G I I S I F F A L G L Y N G
 CTGACTTCTCTCACGACAACggtattATTCTATTTTCTTCGCTTTGGGTTTGTACAACG
 1081 -----+-----+-----+-----+-----+ 1140
 GACTGAAGAGAGTGCTGTTGccataaTAAAGATAAAAGAAGCGAAACCCAAACATGTTGC

CP-18.7CP-19.7

T A P L S T T S V E S I E E T D G Y S S
 GTACTGCTCCATTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTTACTCTt
 1141 -----+-----+-----+-----+-----+ 1200
 CATGACGAGGTAACAGATGATGAAGACAACCTTAGATAACTTCTTTGACTGCCAATGAGAA

A W T V P F A S R A Y V E M M Q C Q A E
 ctgctTGGACTGTTCCATTGctttctAGAGCTTACGTTGAAATGATGCAATGTCAAGCTG
 1201 -----+-----+-----+-----+-----+ 1260
 gacgaACCTGACAAGGTAAGcgaagaTCTCGAATGCAACTTTACTACGTTACAGTTCGAC
 CP-20

K E P L V R V L V N D R V V P L H G C A
 AAAAGGAACCATTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTG
 1261 -----+-----+-----+-----+-----+ 1320
 TTTTCCTTGGTAACCAATCTCAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACAC
 CP-21

V D K L G R C K R D D F V E G L S F A R
 CTGTTGACAAGTTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTTCGCTA
 1321 -----+-----+-----+-----+-----+ 1380
 GACAACTGTTCAACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGAT
 CP-22

S G G N W A E C F A * Eco RI
 GATCTGGTGGTAACTGGGCTGAATGTTTCGCTTAAGAATTCATATA
 1381 -----+-----+-----+-----+-----+ 1426
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21 SEP. 1999

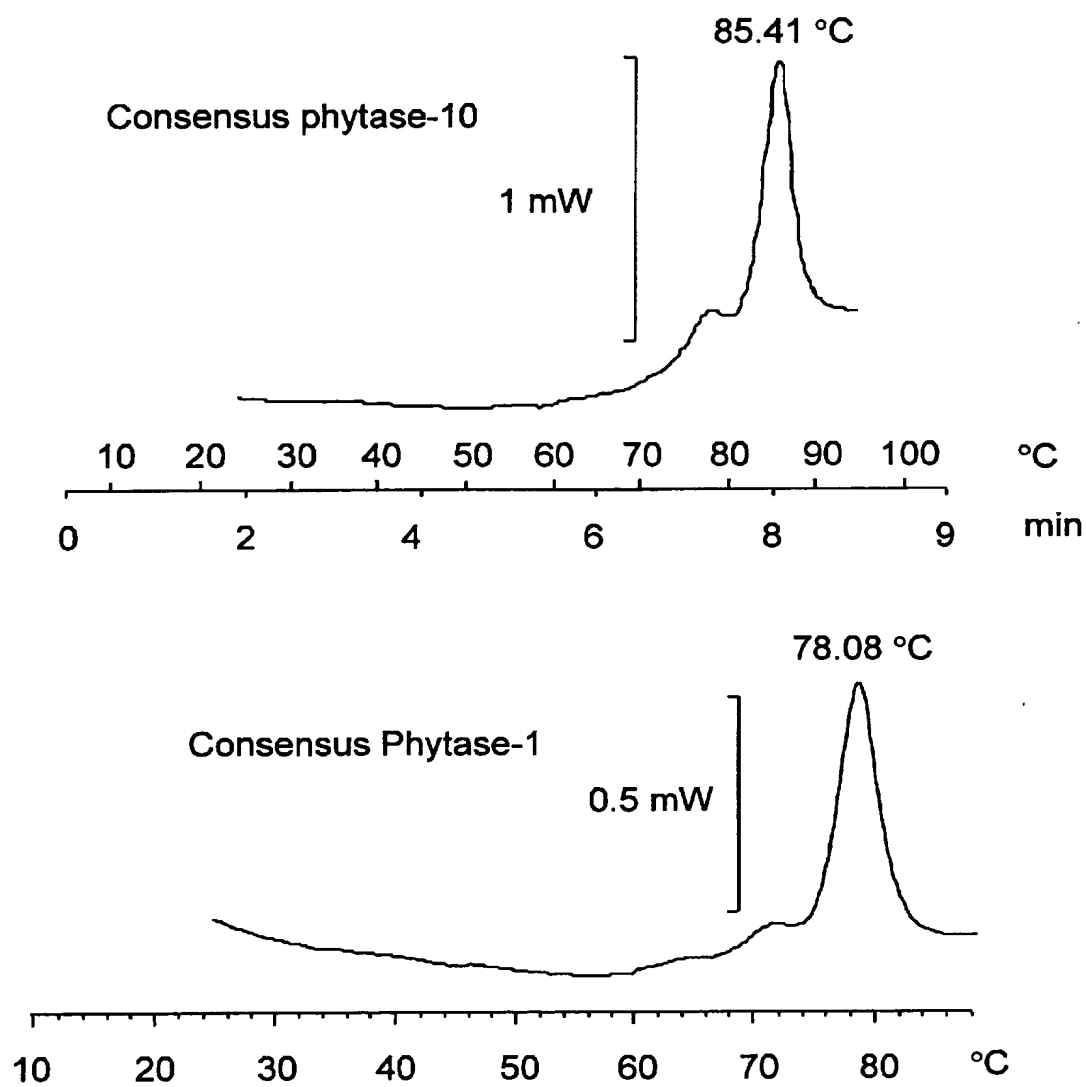
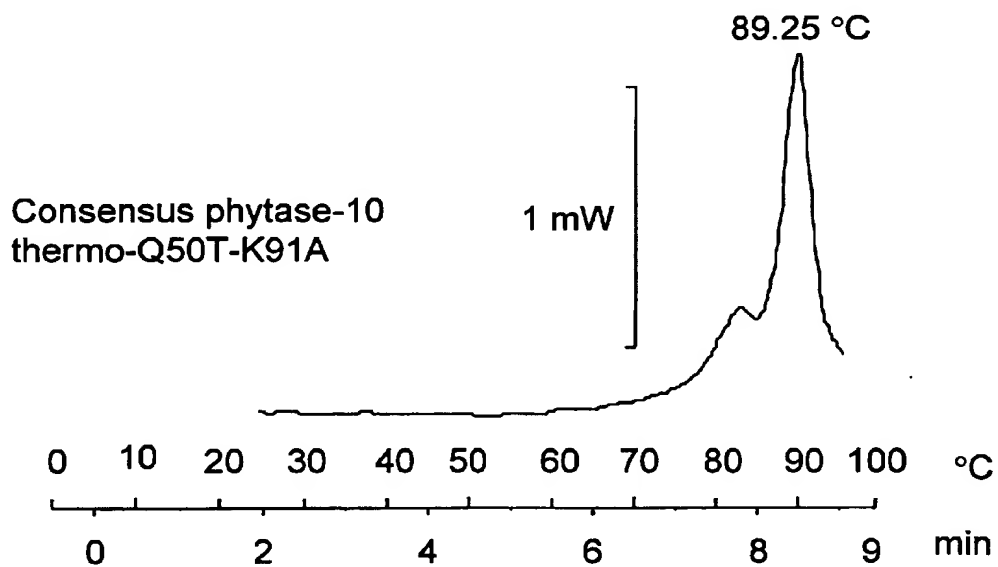
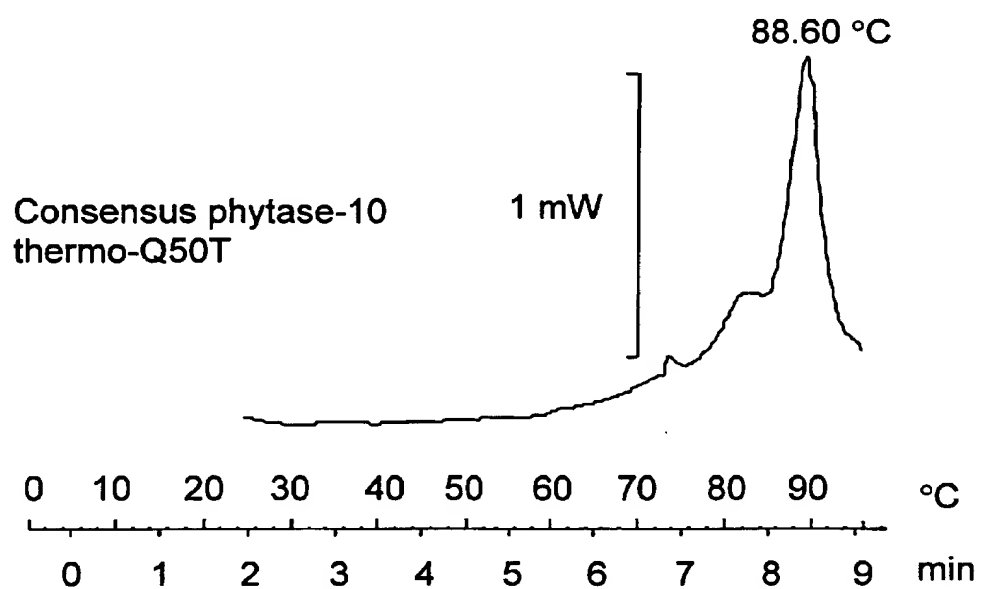
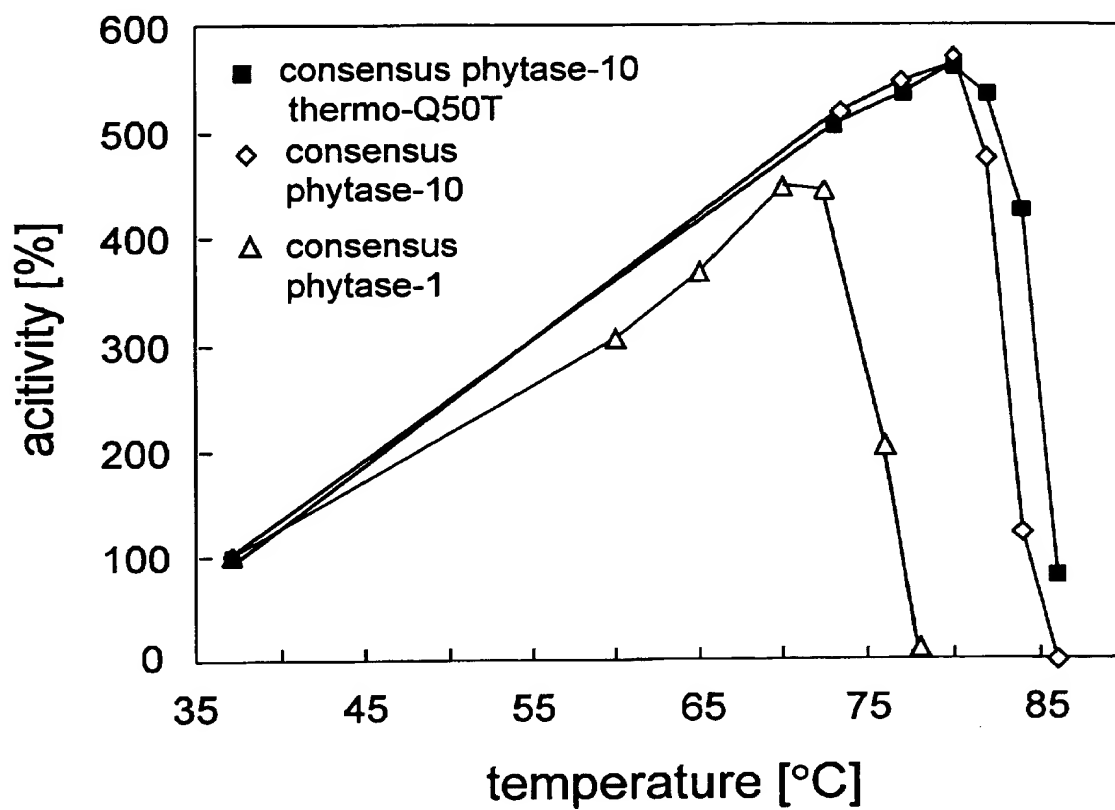
Figure 11

Figure 12

Modtaget PD

2.1 SEP. 1999

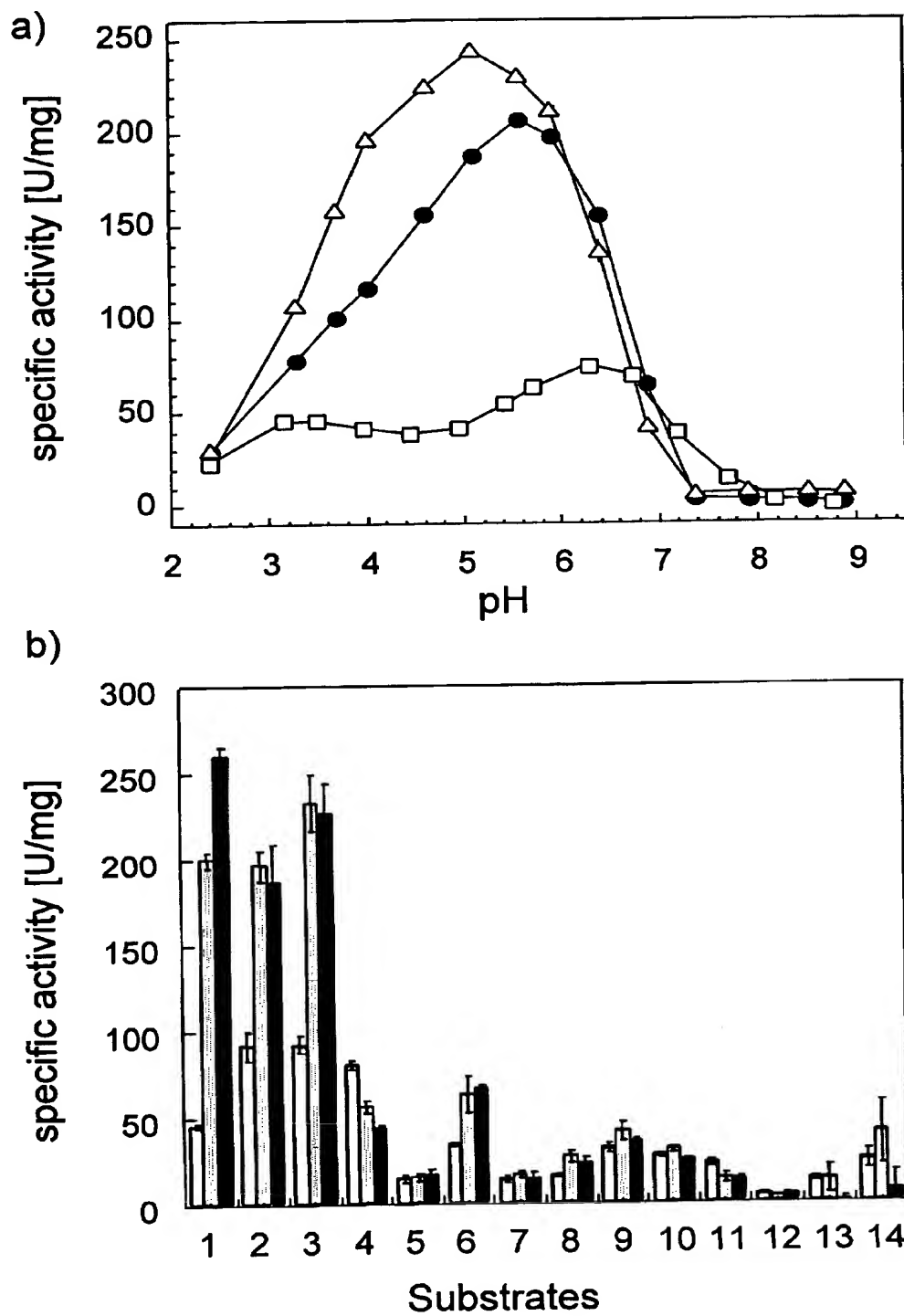
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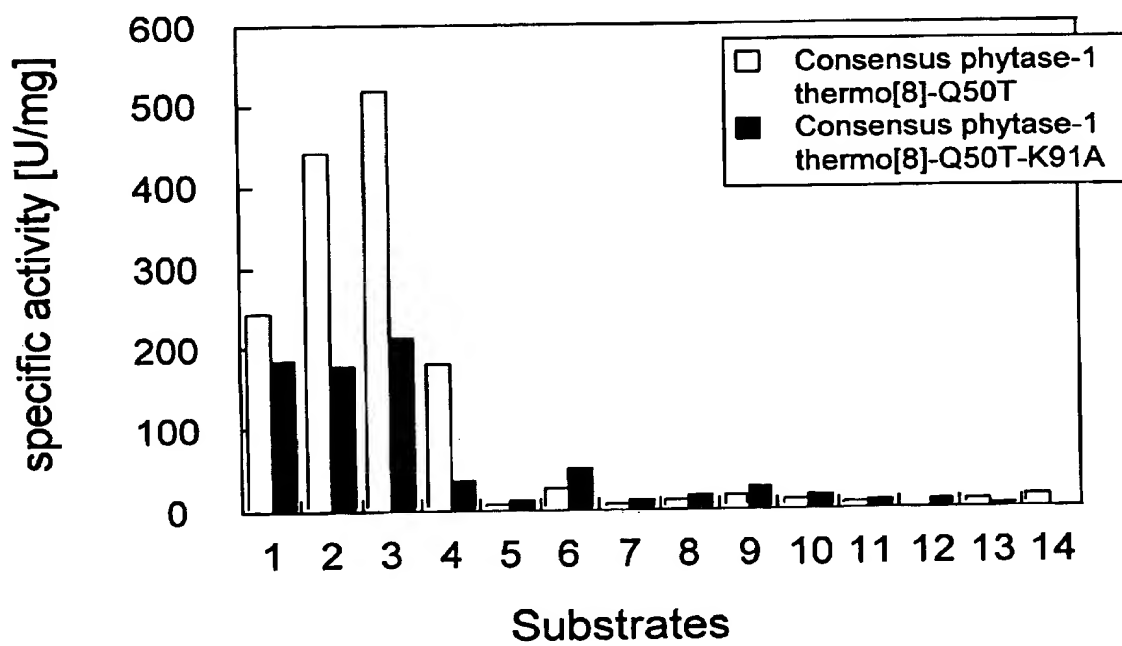
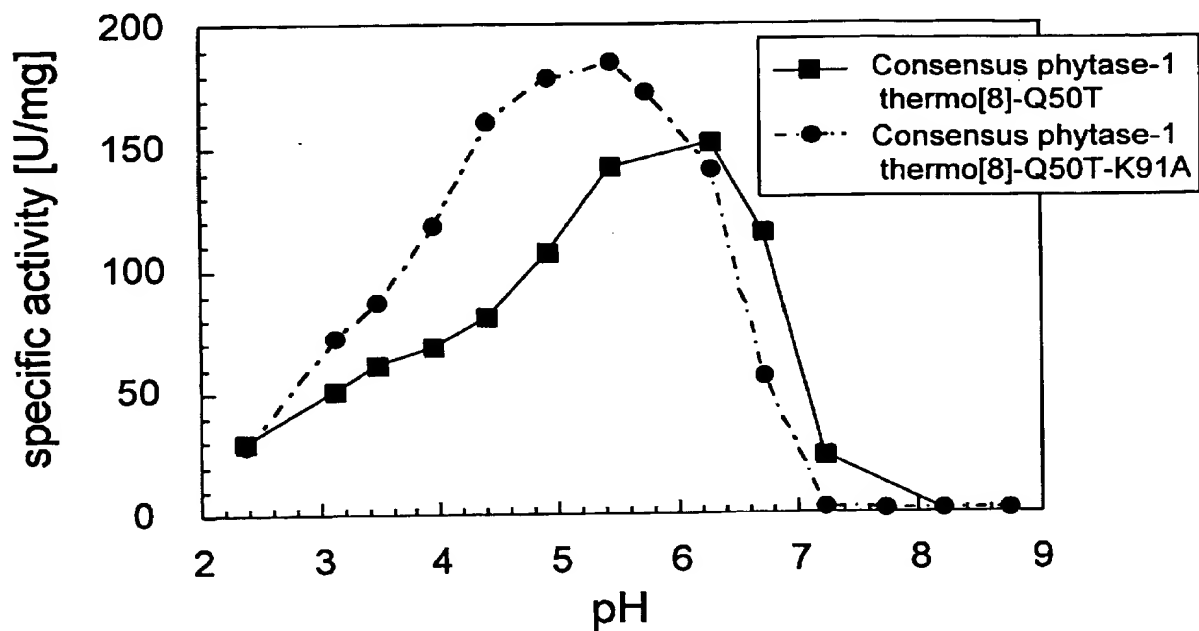
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Figure 14



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21 SEP. 1999

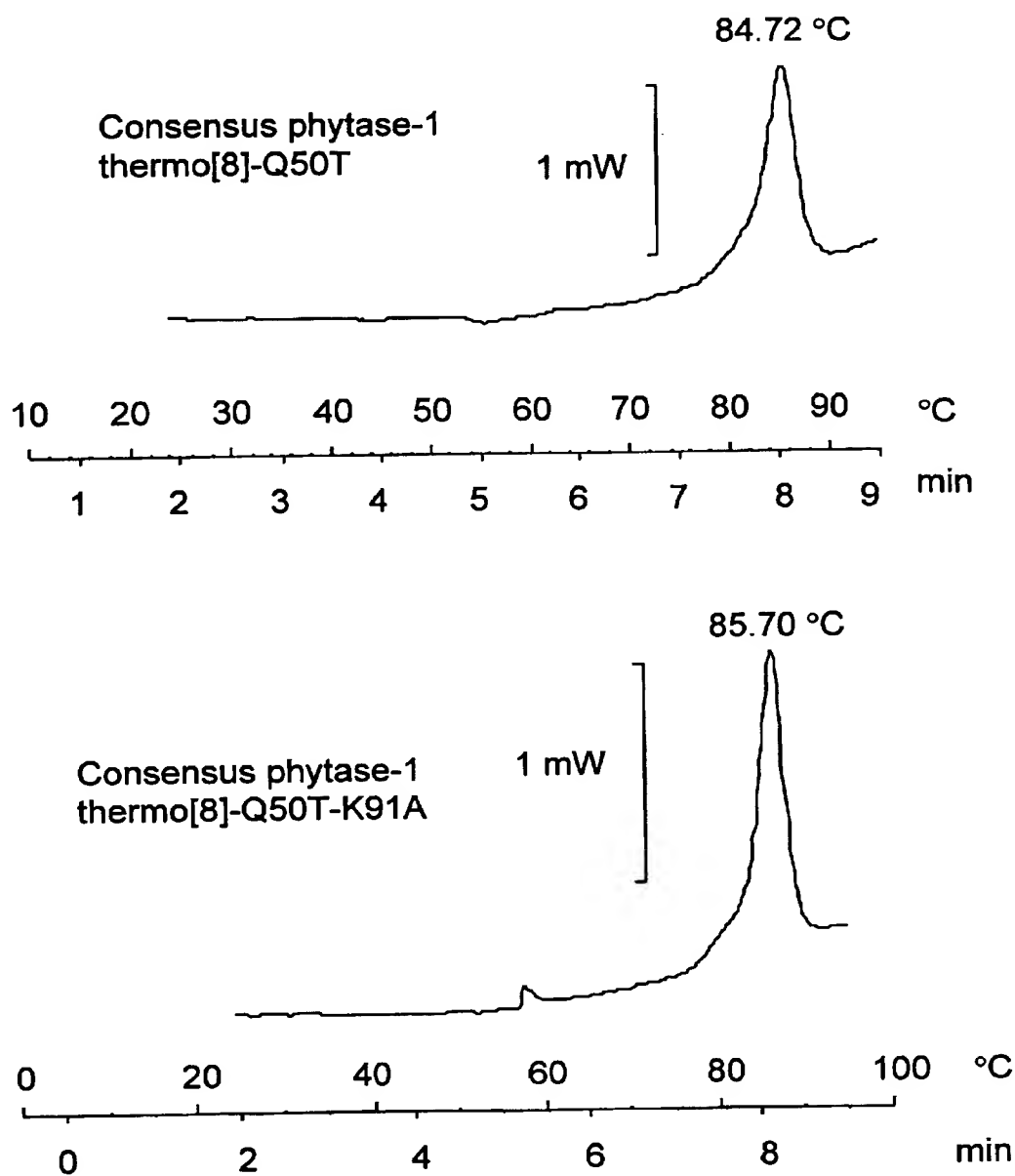
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21 SEP. 1999

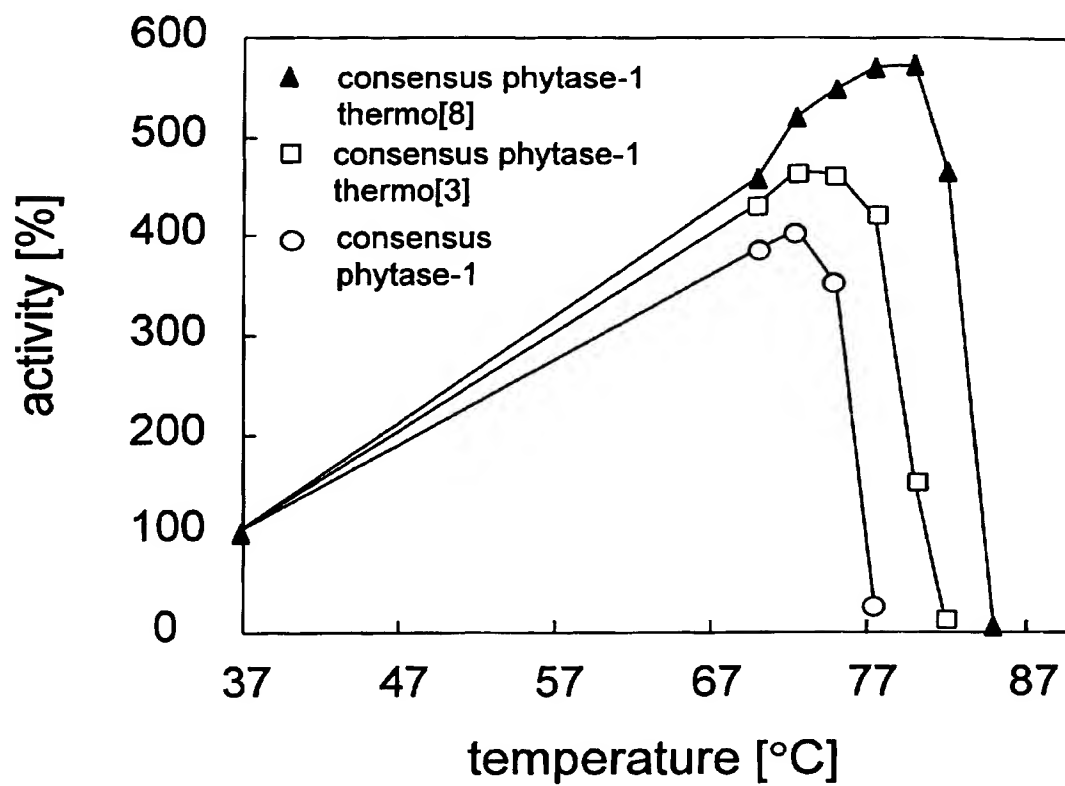
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Figure 16

Modtaget PD

21 SEP. 1999

Figure 17



Modtaget PD

21 SEP. 1999

Figure 18

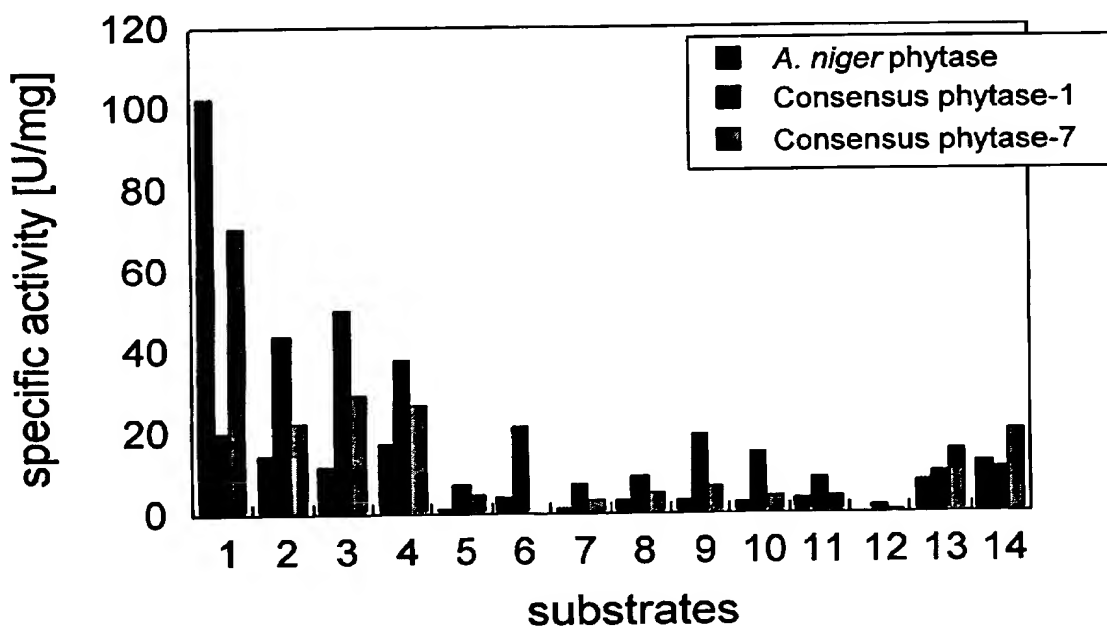
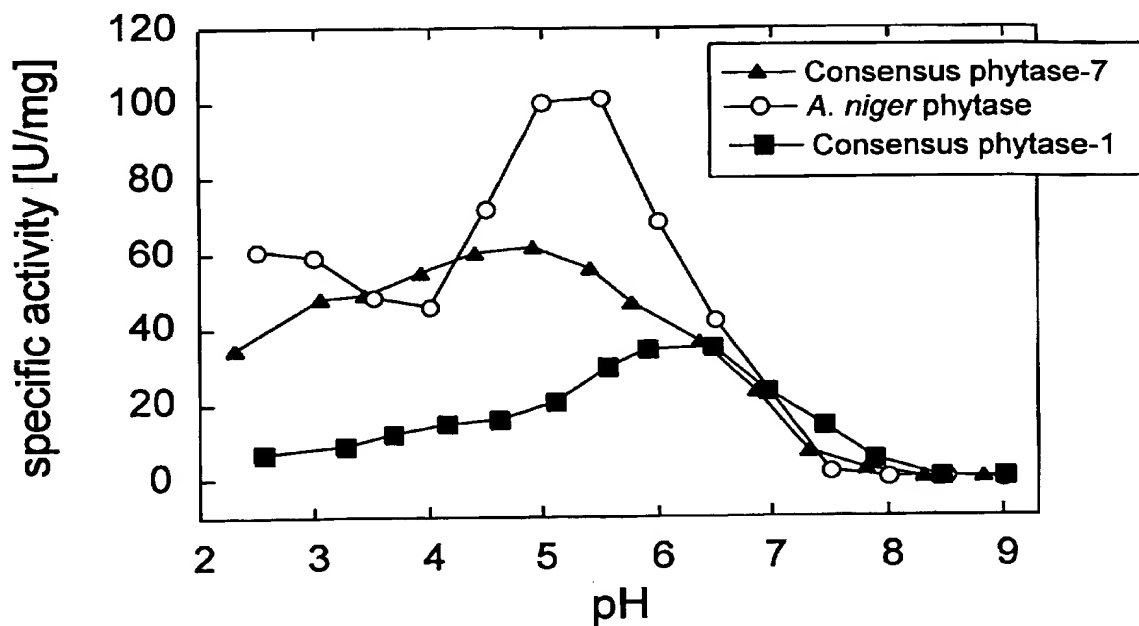


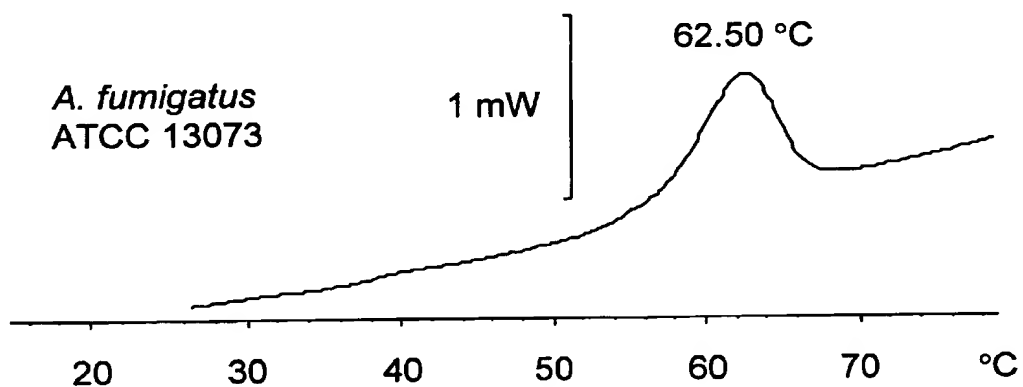
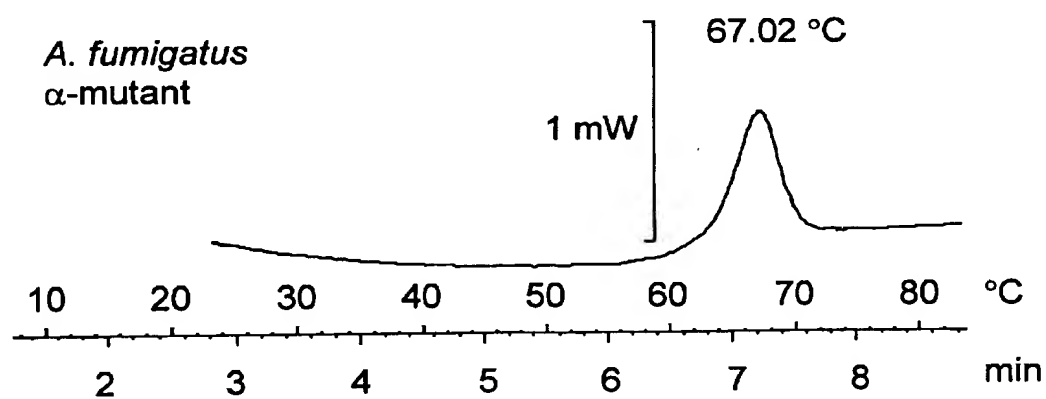
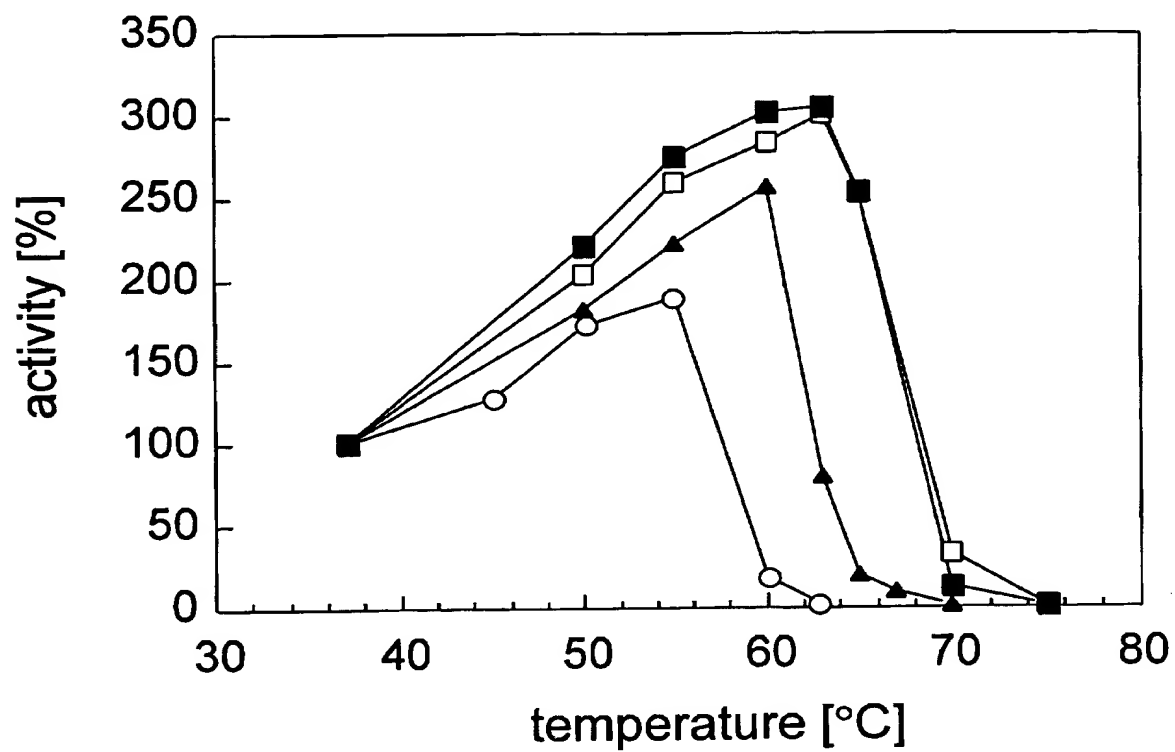
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Figure 20



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Figure 21

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51 YSPYFSLADE SAISPDVPKG CRVTFVQVLQ RHGAREPTSG AATRISALIE
101 AIQKNATAFK GKYAFLKTYN YTLGADDLYP FGANOSSOAG IKFYRRYKAL
151 ARKIVPFIRA SGSDRVIDSA TNWIEGFQSA KLADPGANPH QASPVINVII
201 PEGAGYNNTL DHGLCTAFEE SELGDDVEAN FTAVFAPPPIR ARLEAHLPGV
251 NLTDDEVVNL MDMCPFDIVA RTSDATELSP FCDLFTHDEW IQYDYLGDLD
301 KYYGTGAGNP LGPAQGVGFV NELIARLTHS PVQDHTSTNH TLDSNPATFP
351 LNATLYADFS HDNTMVAIFF ALGLYNGTKP LSTTSVESIE ETDGYSASWL
401 VPFSARMYVE MMQCEAEKEP LVRVLVNDRV VPLHGCGVDK LGRCKRDDFV
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Modtaget PD

21 SEP. 1999